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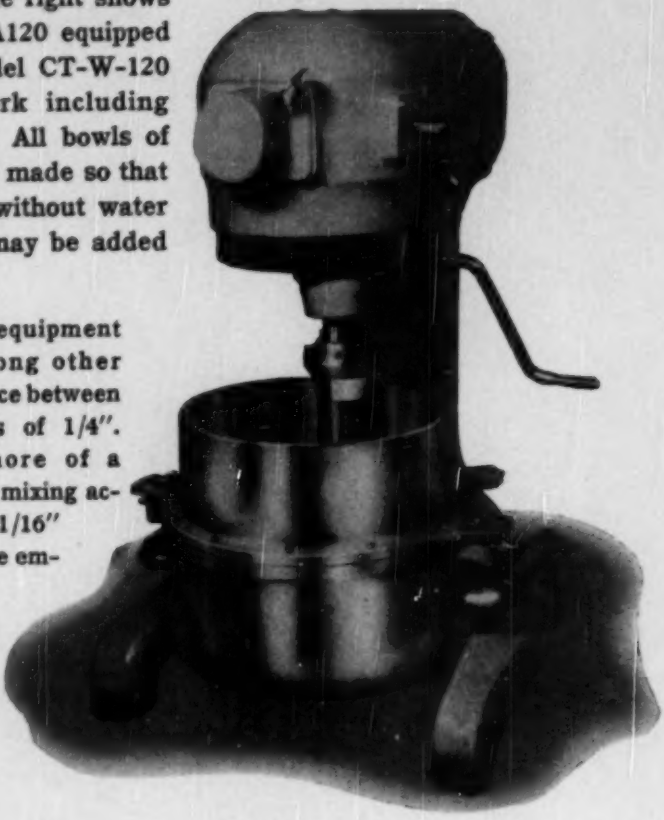

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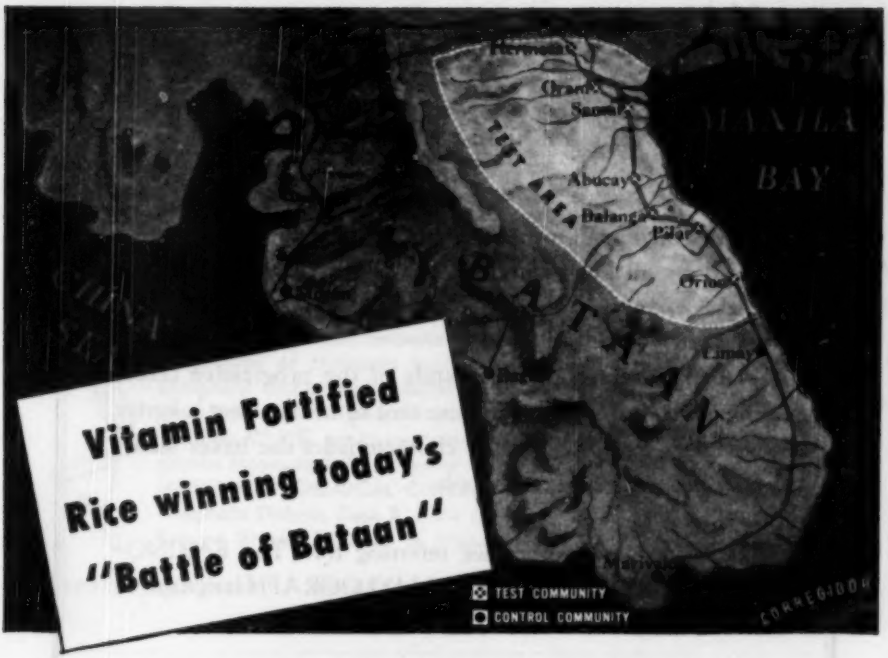

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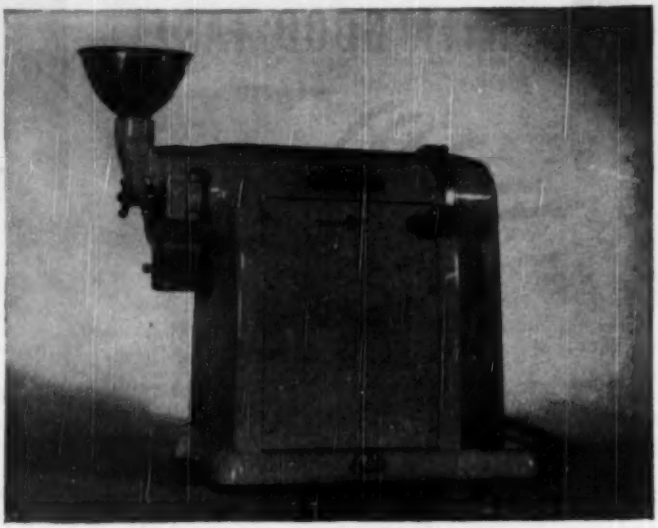

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CEREAL CHEMISTRY

VOL. XXVII SEPTEMBER, 1950 No. 5

STUDIES OF THE EFFECTS OF POLYOXYETHYLENE MONOSTEARATE ON FLOUR AMYLASE ACTIVITY[1,2]

L. F. MARNETT and R. W. SELMAN

ABSTRACT

Polyoxyethylene monostearate increases amylograph maximum paste viscosity of wheat flour pastes to a varying degree, dependent upon the amount of ester added and the alpha-amylase content of the flour. A significant increase in maximum viscosity is produced by polyoxyethylene monostearate in the absence of alpha-amylase. The effect is likewise manifested when the agent is added to pastes made from corn starch and waxy maize.

In amounts less than 0.25% polyoxyethylene monostearate only slightly retarded the dextrinization time with alpha-amylodextrin substrate, but higher percentages prevented complete hydrolysis even after 30 hrs. of incubation.

Maltose production from soluble starch solutions was decreased only when very high levels of amylase were employed in conjunction with abnormally high percentages of polyoxyethylene monostearate.

Favor and Johnson (5), (6), have shown that the inclusion of polyoxyethylene monostearate (hereinafter referred to as POEMS), in the bread formula decreased the rate at which bread crumb became firm in storage. These workers have reported that POEMS has little effect on gas production in fermenting doughs and on proofing.

The experiments reported herein were conducted to determine whether POEMS has any influence on amylase activity under conditions comparable with those which exist in bread making.

The composition of polyoxyethylene monostearate has been described by the aforementioned authors (5). The product used throughout the following investigations had an average molecular weight corresponding to the nonaethylene glycol monostearate.

Procedures and Results

Anker and Geddes (2), Brown and Harrel (3), and Selman and Sumner (10) have descriped the utility of the amylograph in studying

[1] Manuscript received December 23, 1949. Presented at the annual meeting, May, 1949.
[2] Contribution from the C. J. Patterson Company, Kansas City, Missouri.

the pasting characteristics of flour and of various starches. Favor and Johnson (5) employed the amylograph to show the stabilizing effect of POEMS on starch gels.

Effect on Wheat Flour Pastes. The amylograph was used to measure the effect of POEMS on the paste viscosities of malt-supplemented

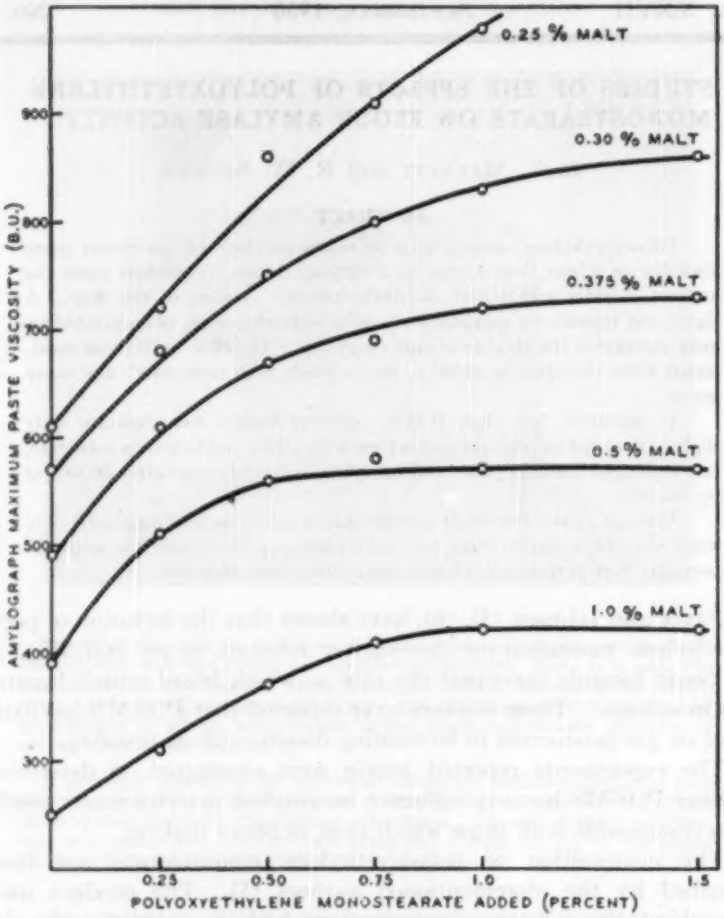

Fig. 1. The inter-relationship of polyoxyethylene monostearate and barley malt with amylograph maximum paste viscosity.

flour since the temperature increase in the amylograph is closely related to that which occurs in dough during the initial stages of baking, when starch conditioning is a major factor. Maximum paste viscosity is regarded as a measure of the starch liquifying effect of alpha-amylase.

Increments of barley malt were added to a commercially milled, non-diastated flour in order to obtain flours of various amylase levels. Varying amounts of POEMS were added to the flour-water suspensions by pipetting appropriate volumes of a 2.5% aqueous POEMS suspension and agitating to obtain adequate dispersion. The amylograph used in these studies had a temperature increase of 1.5°C. per min. The method used was that of Selman and Sumner (10), in which the amylograph is employed to determine starch paste viscosity as a function of thermal gelatinization and amylolysis.

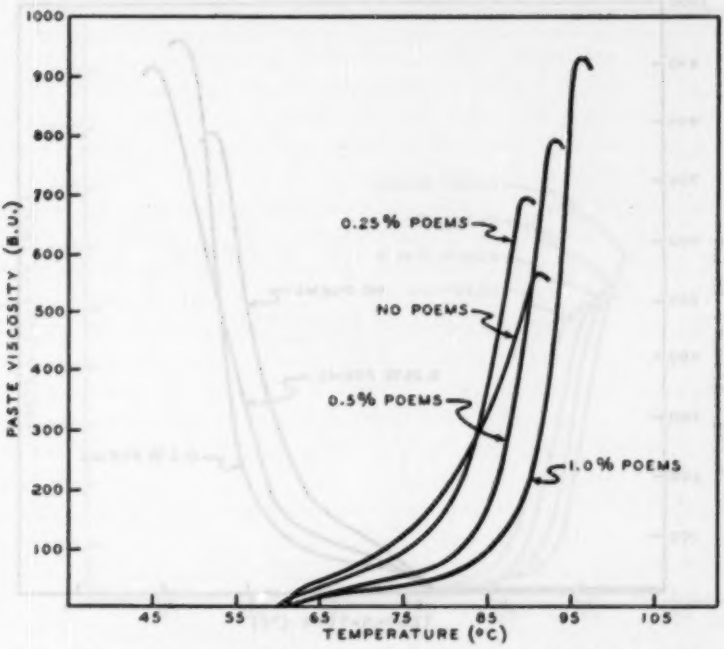

Fig. 2. Effect of polyoxyethylene monostearate on amylograph paste viscosity of a non-diastated flour.

The results shown in Fig. 1 demonstrate that POEMS increased maximum paste viscosity markedly when added in amounts as low as 0.25%. Equivalent increases in maximum viscosity were produced when 0.5% and 0.75% of the ester were present, but the increases with larger additions diminished in magnitude with each additional increment of POEMS.

The increases in maximum paste viscosity produced by POEMS indicate either alpha-amylase inhibition or modification of the starch substrate.

Effect on Non-Diastated Flours. To determine the reason for the viscosity increases observed in the foregoing instances, measurements were made of the pasting properties of unmalted flour. The amylograph procedure employed was again that of Selman and Sumner (10), except that 65 g. of flour were substituted for the customary 100 g., in order that the entire viscosity curve would fall within the recording range of the instrument.

This procedure was repeated in the presence of 20 p.p.m. of mercuric chloride to insure complete inactivation of traces of the amylases (4).

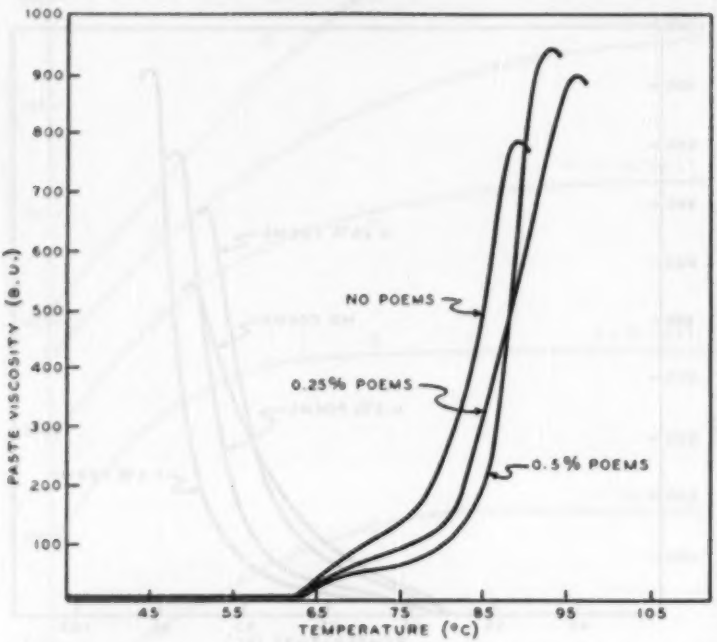

Fig. 3. Effect of polyoxyethylene monostearate on amylograph paste viscosity of a non-diastated flour in the presence of 20 p.p.m. mercuric chloride.

The results (Figs. 2 and 3) suggest that alpha-amylase inactivation was not a major factor, if a factor at all, in the increase in maximum paste viscosity observed earlier; the marked effect in the absence of alpha-amylase indicated a modification of the starch substrate. These curves also indicate that POEMS inhibited granule swelling at temperatures slightly above the transition temperature.

Experiments have shown that POEMS precipitates a portion of the starch from a corn starch gel. Precipitation of this nature may be due to hydrogen bonding of the ester with starch through the terminal hydroxyl group or through the ether oxygens of the polyoxyethylene

chain. Linear organic molecules containing a polar group have long been known to bond with amylose (8) but have not been reported to coordinate with amylopectin. Analogous complex formations are believed to occur between POEMS and starch.

Schoch and French (9) have observed that polar organic compounds possessing both hydrophilic and hydrophobic groupings restrict granule swelling and thereby prevent leaching of the amylose. POEMS has been shown to exert the same effect on the starch of wheat flour in the foregoing studies. This phenomenon may be explained by

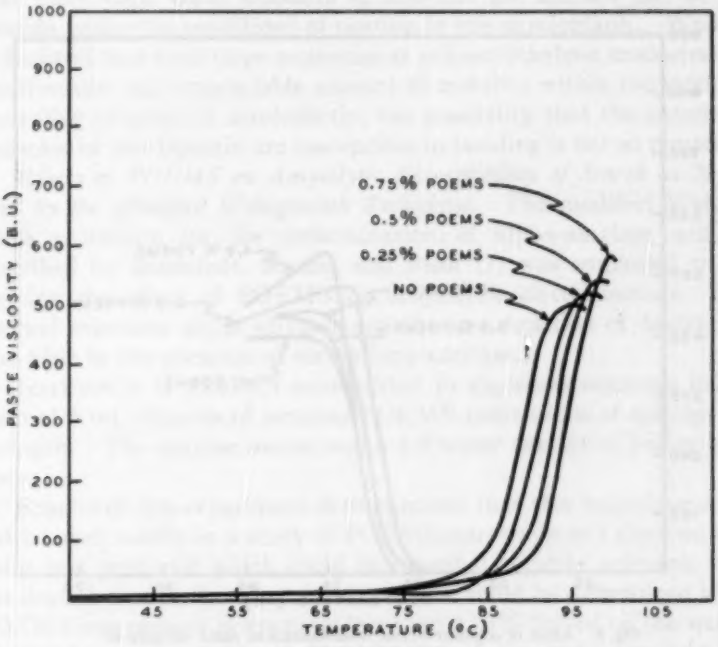

Fig. 4. Effect of polyoxyethylene monostearate on amylograph paste viscosity of commercial corn starch.

assuming the polar group to be oriented with chains of amylose, or possibly with the end branches of amylopectin, near the granule surface. Such an increase in hydrophobic character might well explain the restriction in viscosity of starch pastes at temperatures slightly above the transition temperature. With additional heating in the amylograph, however, the granule may become so distended that the fatty acid chains no longer are able to restrict hydration and a sudden rise in viscosity is manifested. The increase in maximum paste viscosity is possibly caused by an actual increase in granule

diameter through the addition of polyoxyethylene stearate chains; the granule dimensions and mechanical friction are thereby increased. The possibility also exists that restricted distention in the early stages of gelatinization allows less mechanical breakdown of the granule with a consequent higher viscosity in the later stages.

Effect on Corn Starch Pastes. Thirty-six grams of commercial corn starch and 450 ml. of water, with and without increments of POEMS, were pasted in the amylograph to determine the effect of the ester on the viscosity of corn starch pastes. The results in Fig. 4 show that POEMS restricted granule swelling in the initial stages of

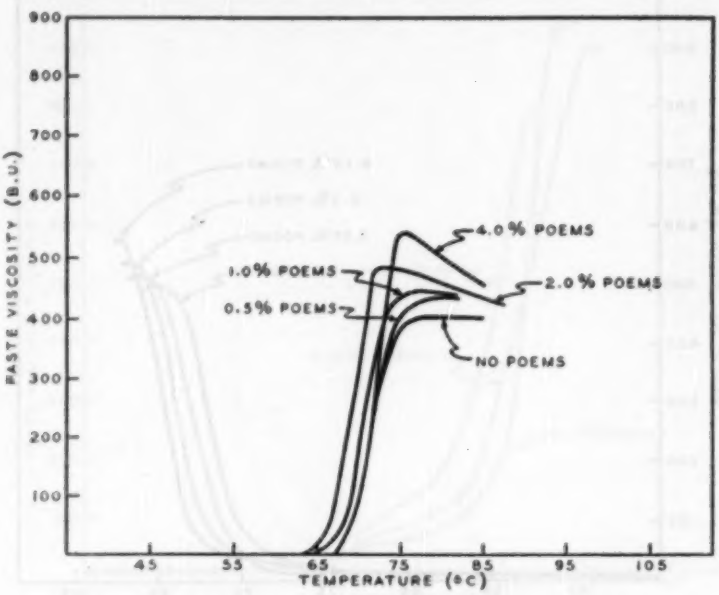

Fig. 5. Effect of polyoxyethylene monostearate on paste viscosity of waxy maize starch.

gelatinization and increased maximum paste viscosity. The magnitude of the increases in corn starch pastes however, were not as great as those observed with wheat flour pastes.

Effect on Waxy Maize Starch Pastes. As a means of determining the effect of POEMS on amylopectin, 32 g. of waxy maize starch were suspended in 450 ml. of water and gelatinized in the amylograph. Increments of POEMS were provided by replacing a portion of the water with an equal volume of a 2.5% POEMS suspension.

As is evident from Fig. 5, POEMS increased the maximum paste viscosity of waxy maize starch pastes, but the effect was less pro-

nounced than that produced on either corn starch or wheat flour. Gelatinization temperatures were unaffected, and the inhibition of granule swelling in the early stages observed in the instances of corn and wheat starch, was noticeably absent. These results suggest the possibility that POEMS may bond with amylopectin, but to a lesser extent than with corn or wheat starch. While waxy maize starch contains traces of amylose, this fraction is apparently tightly bound within the granule since a gelatinized waxy maize starch does not produce the blue color with iodine. Response to the iodine test becomes positive only after such a paste is autoclaved. It is not likely that more than trace amounts of amylose are leached out of the granule under the conditions of pasting in the amylograph. While it is doubtful that such large molecules as polyoxyethylene monostearate could exhibit any appreciable amount of mobility within the intricate branching network of amylopectin, the possibility that the outermost branches of amylopectin are susceptible to bonding is not so remote.

Effects of POEMS on Amylolytic Susceptibility of Starch as Measured by the Modified Wohlgemuth Technique. The modified Wohlgemuth procedure for the determination of alpha-amylase activity described by Sandstedt, Kneen, and Blish (7) was employed to determine the effect of POEMS on amylolytic dextrinization. This method measures alpha-amylase activity as a function of dextrinization time in the presence of excess beta-amylase.

Increments of POEMS were added to digestion mixtures in the form of 5 ml. aliquots of aqueous POEMS suspensions of appropriate strength. The enzyme source was a 2:5 water extract of barley malt flour.

Results of this experiment demonstrated that this technique could not be used validly in a study of POEMS-starch systems since no end-point was produced which could be regarded as truly achromic with the dextrin standard. Dextrinization time could be determined when POEMS was present in amounts less than 0.25% (based on the weight of soluble starch used in the preparation of the alpha-amylodextrin substrate) and little effect on alpha-amylase activity could be detected at these levels. At higher percentages the dextrin end-point was obscured by the presence of a blue POEMS-amylose-iodine precipitate which was detectable even after 30 hrs. of amylolysis. That POEMS precipitates a portion of the amylose and thus renders it not susceptible, or at least much less susceptible, to amylolytic hydrolysis, is established. It was likewise noted that POEMS precipitated portions of the alpha-amylodextrin substrate during incubation.

If the blue amylose-iodine precipitate be allowed to settle to the bottom of the test tube, the supernatant may be observed as a dextrin

red-brown color and the sample may then be regarded in terms of dextrinization. However, no attempt was made to make a quantitative measure of dextrinization time after settling of the precipitate, since the time involved would depend upon the amount of susceptible substrate remaining, the quantity of which would vary with the percentage of POEMS used.

Effect of POEMS on Maltose Production. To determine the effect of POEMS on production of maltose from starch, the following method

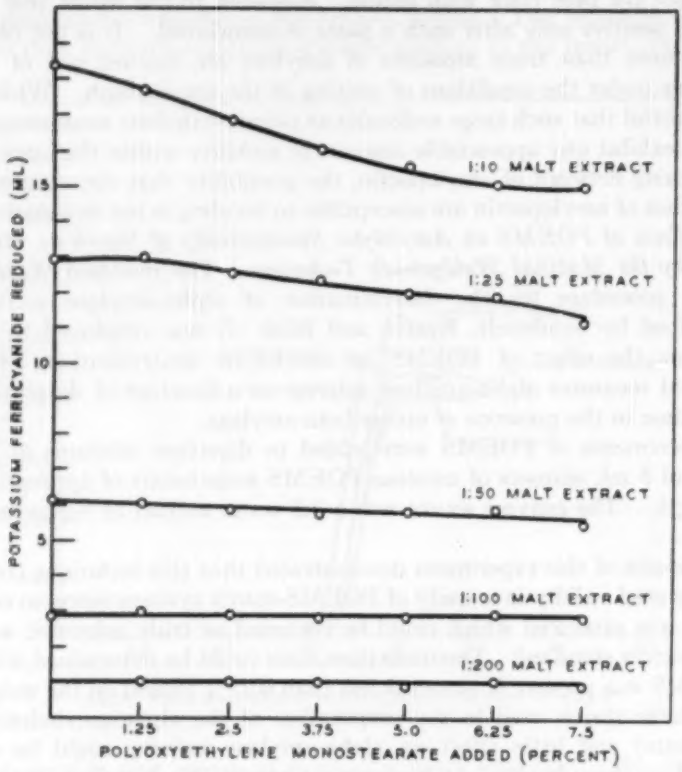

Fig. 6. The effects of polyoxyethylene monostearate on maltose production.

was applied. A mixture of the substances indicated below was incubated for 15 min. at 30°C. ± 0.5°C.:

(1) Distilled water	} to total	9 ml.
(2) POEMS—indicated increment of 1.0% suspension		
(3) Buffered 2.0% Lintner soluble starch		20 ml.
(4) Malt extract—indicated strength		1 ml.
	Total	30 ml.

POEMS was added to the soluble starch immediately before the addition of malt extract. Following the incubation period 10 ml. of 0.5 *N* sodium hydroxide were added to the sample, and a 5 ml. aliquot taken for the determination of reducing sugars. The potassium ferricyanide oxidation procedure as described in *Cereal Laboratory Methods* (1) for measuring diastatic powers of malt was used for determining the maltose produced. Blank tests were made for each series of determinations.

The data given in Fig. 6 demonstrate that POEMS affects maltose production only when present in high percentages in conjunction with very high malt levels. It appears that the observed reduction in maltose produced at high levels of malt and POEMS is not attributable to enzymatic inhibition but rather to a reduction in the quantity of susceptible substrate present. It is concluded from these data that maltose production in fermenting doughs is probably unimpaired by the levels of POEMS commonly employed.

Discussion

Since the foregoing experiments were preliminary in scope, the results suggest several aspects which warrant future investigation. Without doubt, a study of the effect of POEMS on the individual starch fractions warrants additional experimentation. The affinity of the agent for amylopectin, both in the presence and in the absence of amylose, should be determined. The results of any studies relating the starch fractions to the problem of bread staling must be interpreted in such a way as to allow for the possibility that the firmness retarding mechanism of POEMS involves primarily its action on the entire granule. Whether starch gelatinization presupposes complete rupture of its structure or only extreme distention has been discussed by Schoch (8) who regards the latter viewpoint to be "eminently reasonable." It is doubtful that granules are completely disrupted in the baking of bread, and hence this factor must be considered in any study dealing with the influence of starch fractions.

Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (5th ed.). Am. Assoc. Cereal Chemists, St. Paul, Minnesota (1947).
2. Anker, C. A., and Gedoes, W. F. Gelatinization studies upon wheat and other starches with the amylograph. Cereal Chem. **21**: 335–360 (1944).
3. Brown, R. O., and Harrel, C. G. The use of the amylograph in the cereal laboratory. Cereal Chem. **21**: 360–369 (1944).
4. Euler, H., and Pope, T. H. General chemistry of the enzymes. p. 116. John Wiley and Sons, New York (1912).
5. Favor, H. H., and Johnston, N. F. Effect of polyoxyethylene stearate on the crumb softness of bread. Cereal Chem. **24**: 346–355 (1947).
6. Favor, H. H., and Johnston, N. F. Additional data on effect of polyoxyethylene stearate on the crumb softness of bread. Cereal Chem. **25**: 424–425 (1948).

7. SANDSTEDT, R. M., KNEEN, E., and BLISH, M. J. A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. 16: 712–723 (1939).
8. SCHOCH, T. J. The fractionation of starch. Advances in Carbohydrate Chemistry (edited by W. W. Pigman and M. G. Wolfram) 1: 247–277. Academic Press, Inc., New York (1945).
9. SCHOCH, T. J., and FRENCH, D. Studies on bread staling. I. The role of strach. Cereal Chem. 24: 231–249 (1947).
10. SELMAN, R. W., and SUMNER, R. J. The use of the amylograph for flour malt control. Cereal Chem. 24: 291–299 (1947).

THE INFLUENCE OF DRYING TEMPERATURE AND DRY ICE TREATMENT ON THE QUALITY OF IMMATURE WHEAT[1]

MAX MILNER AND JOHN A. JOHNSON

ABSTRACT

Drying immature hard red winter wheat from a moisture value of 50 to 14% moisture content at 40 F. caused a reduction in maltose value and in alpha- and beta-amylase activity in comparison with grain dried at room temperature. The alpha-amylase activity of this very immature wheat which was dried at room temperature was much greater than that of similar samples harvested subsequently at lower moisture values and handled in the same manner. A smaller reduction in maltose value occurred when immature grain with 26.5% moisture was dried at 50 F. Dry ice treatment had little effect on several of the biochemical properties of wheat although germination was damaged when grain moisture was high. Loaf volume and crumb grain were affected adversely by dry ice treatment of wheat and these effects appeared to be due to the carbon dioxide rather than to the low temperature in the grain caused by the dry ice.

The influence of harvest conditions and storage treatment on the milling and baking quality of immature or freshly harvested wheat is generally believed to be important, yet meager information on these factors exists. Swanson (5) found that an elevated maltose value was obtained by drying immature wheat in the shock at low temperatures (50°F.). It was desired to re-examine these results and to study other chemical and physical factors which might be affected by drying conditions.

Considerable interest existed during the harvest seasons of 1948 and 1949, particularly in the southwest wheat producing area, concerning the use of dry ice (solid carbon dioxide) in stored grain to destroy insects and decrease grain temperature and respiration. No information was at hand regarding the influence of such treatment on wheat and flour quality and it seemed of interest therefore to initiate studies along this line.

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Materials and Methods

Preparation of Wheat Samples. A plot of hard red winter wheat (Pawnee variety) on the Kansas State College Agronomy Farm was selected. The weather was warm and sunny throughout the filling and ripening period following initiation of harvesting on June 14, 1949, with the exception of one light shower on June 22. The sample harvested by hand on June 14, contained 50% moisture, that taken on June 17, 26.5% moisture and a final sample of fully mature grain harvested by combine on June 24 had a moisture content of 14.0%. On the day prior to the shower on June 22, the moisture content of the wheat in the field was 13.0%.

The following treatments were accorded the various wheat samples.

Sample No. 1. Wheat heads harvested by hand on June 14 when the grain was in the dough stage and contained 50% moisture, were threshed with a small nursery thresher. Two portions of this sample were treated separately as follows: (a) A portion of the fresh sample was spread out immediately to dry on a laboratory bench at about 80°F. with the aid of an electric fan. (b) Immediately after harvest a sample was sealed in a flask and held in a refrigerator at 40°F. for 48 hours. It then was spread out thinly to dry in the refrigerator at this temperature.

Sample No. 2. The grain obtained on June 17 containing 26.5% moisture was dried to 12% moisture on a laboratory bench with the aid of an electric fan. Prior to milling, the wheat was reconditioned to 15% moisture.

Sample No. 3. Same original grain as Sample 2 was spread out thinly for drying in the cold room at 50°F. After five days the grain had a moisture value of 14.0%. Prior to milling, this wheat was reconditioned to 16% moisture.

Sample No. 4. Same original grain as Sample 2, air-dried in the laboratory to 16% moisture and then milled immediately.

Sample No. 5. Same original grain and drying treatment as Sample 4, but before milling, treated with 2% of coarsely granulated dry ice in a tape-sealed can insulated with burlap and cardboard. Sample was held in this manner for 48 hours before the grain was milled. All the following samples which received dry ice treatment were handled in the same manner.

Sample No. 6. Same as Sample No. 5 but treated with 20% of dry ice.

Sample No. 7. Original grain with 26.5% moisture was dried in the laboratory to 12.5% moisture and treated with 2% of dry ice for 48 hours, followed by reconditioning to 16% moisture content prior to milling.

Sample No. 8. Same as Sample 7, but treated with 20% dry ice.

Sample No. 9. Original grain dried as Samples 7 and 8, reconditioned to 16% moisture prior to treatment with 2% dry ice.

Sample No. 10. Same as Sample 9 but treated with 20% dry ice.

Sample No. 11. Original grain (26.5% moisture) was treated with ⅓ of its weight in dry ice. The sample then was air-dried for 14 hours to 15% moisture and milled.

Sample No. 12. The original grain was air-dried in the laboratory for 12 hours to a moisture content of 18.5% and then stored in a sealed glass jar which had been flushed with nitrogen, in the laboratory deep freeze unit at −20°F. The jar was removed after 24 days, and after being warmed to room temperature the grain sample was air-dried to 15% moisture and milled.

Sample No. 13. Fully mature, field-ripened wheat, harvested June 24, with a moisture value of 14%.

Chemical Determinations on Wheat and Flour. The following determinations were carried out on the wheat samples in accordance with methods outlined in Cereal Laboratory Methods, 5th edition: (a) moisture content, (b) protein content, (c) ash content, and (d) maltose value.

Saccharifying activity and free and total alpha- and beta-amylase activity were determined by the methods of Kneen and Sandstedt (3) and Kneen, Miller and Sandstedt (2).

Additional determinations on flour included (a) water absorption determined with the farinograph by titration to the 500 unit line, (b) maximum viscosity of flour-water paste in the amylograph using 65 g. of flour and 450 ml. of water, and (c) baking test employing a commercial sponge-dough procedure with and without potassium bromate (Johnson and Miller, 1).

Germination values obtained by the normal procedure with and without pre-chilling the seed were supplied by the Kansas State Seed Laboratory.

Milling was carried out with the Buhler experimental mill. Extraction varied from 68 to 70%.

Results

Influence of Drying at Low Temperature on Properties of Immature Wheat. The analytical results obtained on the very immature wheat (50% moisture) appear in Table I together with data for field-matured wheat harvested later from the same plot.

The very immature wheat was considerably higher in maltose value and in free and total alpha-amylase activity than mature grain. These results confirm those of Schwimmer (4). Drying the immature wheat

TABLE I

INFLUENCE OF DRYING TEMPERATURE ON MALTOSE VALUE AND AMYLASE ACTIVITY OF VERY IMMATURE WHEAT. (WHEAT CONTAINED 50% MOISTURE AT HARVEST)

Treatment of wheat	Maltose value	Saccharifying activity[1]		Beta-amylase activity[2]		Alpha-amylase activity[2]	
		Free	Total	Free	Total	Free	Total
Dried at room temperature	276	0.78	0.82	0.73	0.77	0.46	0.55
Dried at 40°F.	133	0.59	0.66	0.58	0.65	0.05	0.10
Field-matured wheat	204	0.63	0.70	0.63	0.68	<0.02	—

[1] Grams starch converted to sugar at 30° C. by 1 g. sample in one hour.
[2] Grams starch dextrinized by 1 g. sample in one hour at 30° C. in presence of excess beta-amylase.

at low temperature significantly reduced the maltose value in comparison with similar wheat dried at room temperature. This latter result is quite opposite to that recorded by Swanson (5) who noted a considerable increase in maltose values when wheat in the shock was dried in a room at 50°F. in comparison with the maltose value of the same grain which had been dried in the sun. Drying at low temperature

TABLE II

BIOCHEMICAL PROPERTIES OF FRESHLY HARVESTED WHEAT AS INFLUENCED BY DRYING AND STORAGE AT LOW TEMPERATURE AND BY TREATMENT WITH DRY ICE

Sample no.	Treatment	Maltose value	Germination		Saccharifying activity[1]		Beta-amylase activity[2]		Alpha-amylase activity[2]	
			Regular	Prechill	Free	Total	Free	Total	Free	Total
			%	%						
2	Dried to 12% moisture at room temp., reconditioned	166	64	70	0.62	0.71	0.62	0.70	<0.02	0.05
3	Dried 50°F.	140	66	65	0.63	0.69	0.63	0.69	<0.02	<0.02
4	Dried to 16% moisture	170	81	90	0.64	0.70	0.64	0.69	<0.02	<0.02
5	Dried to 16% moisture, 2% dry ice	174	62	60	0.64	—	0.64	—	<0.02	<0.02
6	Dried to 16% moisture, 20% dry ice	172	71	54	0.65	0.72	0.65	0.71	<0.02	<0.02
7	Dried, 2% dry ice, reconditioned	170	65	76	—	—	—	—	—	—
8	Dried, 20% dry ice, reconditioned	157	61	76	—	—	—	—	—	—
9	Dried, reconditioned, 2% dry ice	—	—	—	—	0.70	—	0.69	<0.02	<0.02
10	Dried, reconditioned, 20% dry ice	—	—	—	—	0.69	—	0.69	<0.02	<0.02
11	Fresh damp grain, 33% dry ice	187	20	30	0.64	0.70	0.64	0.71	<0.02	<0.02
12	18.5% moisture, 24 days at −20°F.	—	63	—	0.64	0.69	0.63	0.69	<0.02	0.04
13	Field ripened wheat	204	91	91	0.63	0.70	0.63	0.68	<0.02	<0.02

[1] Samples 2 to 12 inclusive contained 26.5% moisture at harvest. Sample 13 was field ripened wheat containing 14.0% moisture.
[2] Grams starch converted to sugar at 30°C. by 1 g. sample in one hour.
[3] Grams starch dextrinized by 1 g. sample in one hour at 30°C. in presence of excess beta-amylase.

also reduced the saccharifying activity and alpha- and beta-amylase activity. These same properties remained high in the grain dried at normal temperatures. These values decreased with the normal maturation process, as indicated by the analyses for the normal field-matured wheat harvested at a later date.

The Properties of Freshly Harvested Wheat and of the Flour and Bread Produced from It, After Dry Ice Treatment. The effects on a number of wheat properties, of various treatments including drying, storage and dry ice treatment are summarized in Table II.

With one exception, no drastic change in germination or biochemical properties occurred in immature wheat subjected to the wide variety of conditions listed in this table. The maltose value of the sample dried at 50°F. was lower than that of the sample dried at room temperature. This agrees with the similar but larger difference found in the previous experiment when the moisture of the grain at harvest was considerably greater.

The effect of dry ice treatment was remarkably inconsequential in view of the drastically low temperatures to which this immature grain was subjected. Samples containing as much as 16% moisture and treated with dry ice in amounts of 20% or more reached temperatures well below −75°F., yet no deleterious effect on germination or biochemical properties resulted. The one significant effect appeared in the grain which contained 26.5% moisture and which was treated with one-third of its weight of dry ice. In this one case a sharp decrease in germination resulted. A sample of the same wheat containing 18.5% moisture and stored for 24 days in a deep-freeze cabinet at −20°F. showed no apparent decrease in germinative capacity.

Pre-chilling prior to the test for germination generally stimulated germination with immature wheat. The fully mature grain apparently had passed through the dormant period, since the normal germination of 91% was not stimulated by pre-chilling. None of these treatments affected the activity of the amylase enzymes as indicated by the data in the last six columns of Table II.

Several chemical properties of the flours produced from the wheat samples listed in Table II appear in Table III and the biochemical properties are shown in Table IV. The moisture content, protein and ash were with few exceptions fairly consistent in this series of flours, indicating that the milling procedure was fairly uniform. The maltose value (Table IV) tended to decrease when immature wheat was artificially dried. Thus the flour from immature wheat which was dried to 12% moisture in the room and reconditioned before milling (Sample No. 2) had a maltose value of 137, whereas the wheat dried only to 16% moisture and milled, produced flour with a maltose value of 179

TABLE III

CHEMICAL ANALYSIS OF FLOUR SAMPLES MILLED FROM WHEAT DRIED AND STORED AT NORMAL AND LOW TEMPERATURES, AND AFTER TREATMENT WITH DRY ICE

Sample no.	Treatment	Moisture	Protein[1]	Ash[1]
		%	%	%
2	Dried to 12% moisture at room temperature, reconditioned	14.1	8.7	0.41
3	Dried 50°F.	14.7	8.8	0.40
4	Dried to 16% moisture	14.9	8.8	0.47
5	Dried to 16% moisture, 2% dry ice	14.5	9.4	0.51
6	Dried to 16% moisture, 20% dry ice	14.6	9.2	0.47
7	Dried, 2% dry ice, reconditioned	14.3	8.8	0.42
8	Dried, 20% dry ice, reconditioned	14.5	8.8	0.42
9	Dried, reconditioned, 2% dry ice	14.8	8.8	0.42
10	Dried, reconditioned, 20% dry ice	14.6	8.7	0.42
11	Fresh damp grain, 33% dry ice	14.2	9.1	0.59
12	18.5% moisture, 24 days at −20°F.	13.8	—	—
13	Field-ripened wheat	14.5	8.5	0.42

[1] 14% moisture basis.

(Sample No. 4). The maltose value of flour from wheat dried at low temperature was somewhat lower than that from wheat dried at room temperature (Sample No. 3).

Treatment of wheat with dry ice appeared to have little effect on the maltose value of the flour milled from it (Samples 5 to 14 inclusive). It is notable that the flour obtained from field-ripened wheat (Sample No. 13) had the highest maltose value of all. This wheat, as has been

TABLE IV

BIOCHEMICAL PROPERTIES OF FLOUR SAMPLES MILLED FROM WHEAT DRIED AND STORED AT NORMAL AND LOW TEMPERATURES, AND AFTER TREATMENT WITH DRY ICE

Sample no.	Treatment	Maltose value	Maximum amylograph viscosity	Farinograph absorption[1]	Valorimeter value
2	Dried to 12% moisture at room temperature, reconditioned	137	800	52.6	46
3	Dried 50°F.	133	990	54.7	46
4	Dried to 16% moisture	179	600	57.4	45
5	Dried to 16% moisture, 2% dry ice	152	960	55.5	47
6	Dried to 16% moisture, 20% dry ice	130	930	53.6	51
7	Dried, 2% dry ice, reconditioned	142	960	54.3	47
8	Dried, 20% dry ice, reconditioned	155	920	55.0	46
9	Dried, reconditioned, 2% dry ice	130	950	53.3	46
10	Dried, reconditioned, 20% dry ice	144	900	53.6	46
11	Fresh damp grain, 33% dry ice	134	—	51.2	46
12	18.5% moisture, 24 days at −20°F.	—	960	56.2	48
13	Field-ripened wheat	204	865	58.0	44

[1] 14% moisture basis.

pointed out, was matured in ideally warm weather with little rainfall of consequence.

Values for amylograph viscosity show considerable uniformity with the exception of two samples. Sample No. 2 had a value of 800 units which was somewhat below the average of the group, while Sample No. 4 milled from wheat which had been dried to 16% moisture but otherwise untreated showed a value of only 600. This value appears anomalous in view of the other normal properties of the wheat and flour. The lowest absorption was exhibited by the flour obtained from the wheat stored in the presence of dry ice with 26.5% moisture (Sample No. 11). Valorimeter values appearing in the table show little variation among the different flours.

TABLE V

CHARACTERISTICS OF BREAD PRODUCED FROM VARIOUS WHEATS

Sample no.	Treatment	Loaf volume		Crumb grain	
		No bromate	2 mg. bromate	No bromate	2 mg. bromate
		c.c.	c.c.		
2	Dried to 12% moisture at room temperature, reconditioned	2450	2625	70	70
3	Dried 50°F.	2375	2500	50	75
4	Dried to 16% moisture	2250	2575	60	80
5	Dried to 16% moisture, 2% dry ice	2250	2600	50	75
6	Dried to 16% moisture, 20% dry ice	2200	2550	50	70
7	Dried, 2% dry ice, reconditioned	2150	2500	50	75
8	Dried, 20% dry ice, reconditioned	2225	2500	50	70
9	Dried, reconditioned, 2% dry ice	2225	2475	50	80
10	Dried, reconditioned, 20% dry ice	2225	2525	50	75
11	Fresh damp grain, 33% dry ice	2250	2600	50	75
12	18.5% moisture, 24 days at −20°F.	2225	2425	70	85
13	Field-ripened wheat	2000	2225	65	80

The characteristics of the bread baked from these flours appear in Table V. These data show, surprisingly, that the best loaf volume was produced by the immature wheat dried at room temperature (Sample No. 2), rather than from the field-ripened grain (Sample No. 13). A decrease in loaf volume occurred on drying the immature grain at 50°F. (Sample No. 3) and on milling wheat dried only to 16% moisture prior to milling (Sample No. 4). Dry ice treatment caused a small but significantly uniform reduction in both loaf volume and crumb quality of bread derived from immature wheat (Samples 5 to 11 inclusive). However, loaf volume and internal characteristics were not significantly inferior in the bromated loaves from the dry ice-treated grain when these were compared with the sample dried at room temperature and reconditioned for milling, without preliminary dry ice treatment (Sam-

ple No. 2). It is notable that the field-ripened sample (No. 13) yielded considerably lower loaf volumes from both bromated and unbromated doughs than did the immature wheat regardless of the treatment applied. Sample No. 11 is of particular interest since this was obtained from immature wheat with 26.5% moisture which had been treated with ⅓ of its weight of dry ice for 48 hours, and which reached a temperature below −75°F. The gas-retaining properties of this flour were not harmed to any greater extent than were those of flour from grain treated with dry ice at much lower moisture values, and furthermore the bromate response which it exhibited was excellent. However, the crumb grain of this sample appeared to be damaged to an extent similar to that in the other carbon dioxide-treated samples.

Discussion

These preliminary results indicate that drying very immature wheat at low temperature (40°F.) causes a decrease in the alpha- and beta-amylase activity of the flour. This conclusion is supported by the fact that a reduction in maltose value occurred upon drying wheat harvested at 50% moisture and at 26.5% moisture at low temperatures. In the first case this reduction was shown to be accompanied by differences in free and total saccharogenic activity and in free and total alpha- and beta-amylase activities. No explanation is offered now for the marked difference in the results for maltose value obtained here from those of Swanson (5); and further study of this discrepancy is indicated. In this connection, furthermore, it was shown that flour from immature wheat which had been thoroughly dried prior to reconditioning for milling, had a significantly lower maltose value than flour from the same grain dried only to a moisture value of 16% before milling. Partial explanation of this difference may be found in the data of Schwimmer (4) who noted that the solubility of the beta-amylase of immature wheat was irreversibly reduced by drying the grain.

Treatment of immature wheat with dry ice at considerable concentration (2% and 20%) under conditions which allowed the subliming ice to cool the grain to very low temperatures (below −75°F.) is shown in this study to have little influence on the physical and biochemical properties of the wheat with the exception of a reduction in germination of the sample which contained 26.5% moisture. Experimentation with these high concentrations of dry ice and the use of insulation appear to be justified since, in commercial applications, grain directly contiguous to dry ice would undergo the same treatment accorded in this study to the freshly harvested grain.

The baking study indicated that dry ice treatment caused a reduction in crumb quality as evidenced by coarse and open grain. A small

reduction in loaf volume also occurred due to dry ice treatment, indicating that the gas retention properties of the doughs were affected. That these deleterious effects are not attributable primarily to the low temperature attained by the grain with dry ice treatment, is seen from the fact that this same wheat which had been stored at −20°F. for 24 days with 18% moisture and without dry ice treatment, showed crumb characteristics at a par with flour from immature wheat which had been dried at room temperature without other treatment. The nature of the biochemical damage caused by dry ice to the grain as manifested by poorer grain and volume characteristics was not indicated in this study. This result raises the possibility that carbon dioxide generated by excessive respiration in immature grain might similarly injure wheat quality.

An interesting point in this study is the fact that immature wheat harvested at 26.5% moisture and dried in the room yielded bread of considerably better loaf volume and crumb grain than the same wheat which had been allowed to ripen in the field under apparently ideal maturing conditions. The data obtained in this study are, however, insufficient to indicate the degree of significance of this fact.

Literature Cited

1. Johnson, J. A., and Miller, Byron, S. Studies on the role of alpha-amylase and proteinase in breadmaking. Cereal Chem. 26: 371–383 (1949).
2. Kneen, E., Miller, Byron, S. and Sandstedt, R. M. The influence of temperature on the development of amylase in germinating wheat. Cereal Chem. 19: 11–27 (1942).
3. Kneen, E., and Sandstedt, R. M. Beta-amylase activity and its determination in germinated and ungerminated cereals. Cereal Chem. 18: 237–252 (1941).
4. Schwimmer, Sigmund. Development and solubility of amylase in wheat kernels throughout growth and ripening. Cereal Chem. 24: 167–179 (1947).
5. Swanson, C. O. Effect of harvest conditions on a few quality factors in wheat. Cereal Chem. 13: 79–90 (1936).

FACTORS AFFECTING THE COLOR OF MACARONI. III. VARIETAL DIFFERENCES IN THE RATE OF PIGMENT DESTRUCTION DURING MIXING [1]

G. N. IRVINE, C. A. WINKLER, AND J. A. ANDERSON

ABSTRACT

The rates of pigment destruction during mixing in distilled water, 0.001 *M* cyanide, 30% alcohol, and 0.001 *M* alpha naphthol have been investigated with two series of durum varieties. All varieties behave similarly in the various media. The data clearly indicate the different effects produced by cyanide and alpha naphthol; cyanide inhibits the enzymic reaction, and alpha naphthol acts merely as an anti-oxidant. Good and poor varieties may be characterized by an activity coefficient which is calculated from the rate of oxidation, weighted in terms of the pigment available for oxidation. For a series of 13 durum varieties of widely differing quality, this coefficient gave a good indication of macaroni-making quality. There is good evidence that the enzymic activity is characteristic of a variety, but that it is also influenced by environment.

A prior paper (3) in this series dealt with the reaction responsible for the destruction of the xanthophyll pigments of semolina during mixing. Evidence was reported which indicated that the pigment is oxidized through a coupled reaction simultaneously with the peroxidation of unsaturated fats in the dough by the enzyme lipoxidase. During this work, three major inhibiting agents were found: cyanide ion, alpha naphthol, and alcohol.

The present paper is concerned with two variety studies which were made during the more extensive investigation previously reported. The first study was made primarily to determine whether or not the characteristics of the oxidation reaction in water, cyanide, and 30% alcohol were similar for varieties of differing macaroni-making quality. Quite large samples of material were available and the reaction was investigated over a fairly wide range of mixing times. The second series of varieties was much more extensive than the first but much less material was available. It was felt, however, that the evidence obtained with the first series justified using fewer mixing times to obtain the information that was desired from the second series. This series provided a better opportunity to investigate the extent to which rate of oxidation

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is a varietal characteristic and also to contrast the behavior of cyanide and alpha naphthol as inhibiting agents.

Materials and Methods

The first series of varieties comprised the named varieties Carleton, Mindum, Pelissier, and Golden Ball, and two plant breeder's hybrids designated as Bald Medeah × 1317 and 1742. Each variety represented a composite made up from material grown at the same group of experimental stations in Western Canada. But two of the composites represented the 1945 crop and four the 1946 crop.

The second series contained most of the durum varieties which have, at one time or another, been grown in Western Canada; namely, the standard variety, Mindum, the recent varieties Carleton and Stewart, one of the old red durum varieties Pentad, and nine other varieties (see Table III). Each variety sample was composited from material grown in 1946 at 11 experimental stations across Canada. This series was grown for special tests by the Dominion Laboratory of Cereal Breeding and only small samples could be spared for this investigation.

The material in both experiments was processed, as outlined in a previous paper (3), by the disc method of Cunningham and Anderson (2); 31% absorption was used for samples mixed with distilled water, cyanide or alpha naphthol solutions, and 33% for samples mixed with 30% alcohol. Pigment content was determined on the ground discs by the standard method (1) employing the butyl alcohol solvent in conjunction with an Evelyn colorimeter.

Results and Discussion

Series I. Samples were mixed for intervals from one to ten minutes in distilled water, 0.001 *N* potassium cyanide, and 30% alcohol; the reaction curves are shown in Fig. 1. In general, the varieties all yielded similarly shaped curves for a particular treatment. In distilled water, all varieties with the exception of Carleton yielded a three-stage curve. The Carleton sample exhibited an induction period between the first and second stages which previous evidence (3) has shown to be an absorption effect; studies of other samples of Carleton have shown that this behavior is not typical of this variety, but is probably typical of all varieties at some particular absorption level depending on the sample. With 0.001 *N* cyanide, all varieties exhibited a three-segment curve, five out of six showing the slight reversal of the reaction during the third stage. With 30% alcohol the curves show only two segments; for Carleton the slope of the second segment is slightly negative, and for two of the other varieties there is also some reversal at the longer mixing times.

Discussion of the results will be limited to the first two segments of the reaction curves as it is improbable that the reaction ever proceeds beyond this stage during macaroni processing. The rapid initial portion of the curve has been called the "initial reaction," the following

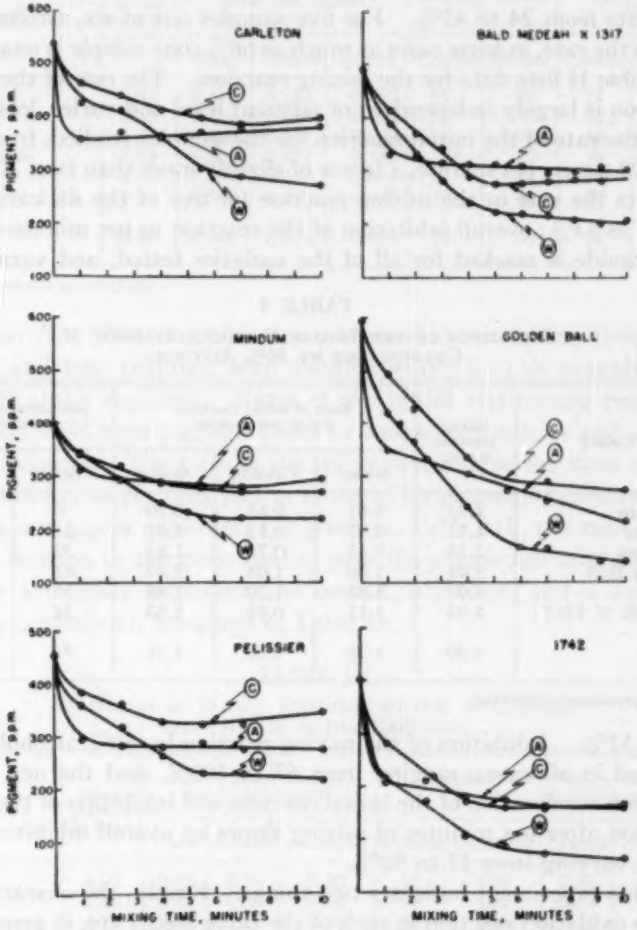

FIG. 1. Loss of pigment during mixing in distilled water (W), 0.001 *N* cyanide (C), and 30% alcohol (A) for six varieties of durum wheat.

zero order segment, the "mixing reaction." Table I gives the rate of the initial reaction for each sample in each of the three media as determined from the reaction curves; the percentage inhibition by cyanide and the percentage inhibition or acceleration by alcohol are calculated from these rates.

Comparison of the initial pigment concentration with the rate of the initial reaction (Table I) shows that the rate of reaction is largely independent of pigment level. The rate of the initial reaction varies over a considerable range—from 0.61 to 1.87 p.p.m. per minute, a factor of three; for all but one sample, which is unaffected, cyanide inhibits this rate from 24 to 42%. For five samples out of six, alcohol accelerates the rate, in some cases as much as 66%; one sample is unaffected.

Table II lists data for the mixing reaction. The rate of the mixing reaction is largely independent of pigment level and varies, less widely than the rate of the initial reaction for the samples studied, from 0.235 to 0510 p.p.m. per minute, a factor of slightly more than two. Cyanide inhibits the rate of the mixing reaction for five of the six varieties as much as 53%; overall inhibition of the reaction to ten minutes mixing by cyanide is marked for all of the varieties tested, and varies from

TABLE I

INHIBITION OF THE INITIAL REACTION BY 0.001 *M* CYANIDE AND BY 30% ALCOHOL

Variety	Initial pigment p.p.m.	Rate of initial reaction p.p.m. per minute			Inhibition of initial reaction, %	
		Water	Cyanide	Alcohol	Cyanide	Alcohol
Mindum	4.02	0.61	0.62	0.90	0	48*
Pelissier	4.57	0.97	0.73	1.61	25	66*
Carleton	5.39	1.11	0.75	1.82	32	64*
Golden Ball	5.98	1.80	1.04	2.49	42	38*
1742	4.02	1.87	1.20	1.88	36	0
B. Med. × 1317	4.94	1.11	0.84	1.63	24	47*
Mean	4.90	1.25	0.85	1.61	32	29*

* Percentage acceleration.

27 to 52%. Inhibition of the mixing reaction by 30% alcohol is very marked in all cases, ranging from 67 to 100%, and the net balance between acceleration of the initial reaction and inhibition of the mixing reaction after ten minutes of mixing shows an overall inhibition in all cases, varying from 17 to 60%.

This experiment indicates two things. Firstly, the characteristics of the oxidation reaction in each of the three media are, in general, the same for durum varieties of widely differing macaroni-making quality; the differences in quality show up essentially as differences in the rates of various phases of the reaction. Secondly, it is evident, from a comparison of the rates of reaction in cyanide and 30% alcohol that although both media effect an overall inhibition of the reaction, the mechanism of inhibition is quite different. This difference has been discussed in a previous paper (3).

TABLE II

INHIBITION OF THE MIXING REACTION BY 0.001 *M* CYANIDE AND BY 30% ALCOHOL

Variety	Initial pigment p.p.m.	Rate of mixing reaction p.p.m. per minute			Inhibition of mixing reaction, %		Overall inhibition, %	
		Water	Cyanide	Alcohol	Cyanide	Alcohol	Cyanide	Alcohol
Mindum	4.02	0.310	0.147	0.062	53	80	27	41
Pelissier	4.57	0.310	0.150	0.015	52	95	52	23
Carleton	5.39	0.300	0.147	−0.033	51	100	48	41
Golden Ball	5.98	0.501	0.527	0.150	5*	70	29	17
1742	4.02	0.330	0.369	0.095	12*	74	31	29
B. Med. × 1317	4.94	0.500	0.400	0.040	20	92	30	32
Mean	4.90	0.365	0.284	0.071	22	80	37	35

* Percentage acceleration.

Series II. These samples were processed at two mixing times, one minute and four minutes, with distilled water, 0.01 *N* cyanide, and 0.001 *M* alpha naphthol. Rates of the initial and mixing reactions were calculated from pigment losses for zero to one minute and for one to four minutes. The rates of the *initial* reactions in the three media, and inhibition as per cent and in terms of its pigment equivalent, for cyanide and alpha naphthol, are given in Table III; the rate of the *mixing* reaction in the three media, with the percentage inhibition by cyanide and alpha naphthol, the overall inhibition, and a series of activity coefficients, are given in Table IV.

TABLE III

INHIBITION OF INITIAL REACTION BY 0.01 *N* CYANIDE AND 0.001 *M* ALPHA NAPHTHOL

Variety	Initial pigment (p.p.m.)	Rate of initial reaction (p.p.m. per minute)			Inhibition %		Pigment equivalent of inhibition (p.p.m.)	
		Water	Cyanide	α-naphthol	Cyanide	α-naphthol	Cyanide	α-naphthol
Carleton	4.91	0.82	0.56	0.00	32	100	0.26	0.82
Mindum	4.40	0.82	0.39	0.00	52	100	0.43	0.82
Goose	4.20	0.63	0.38	0.00	40	100	0.25	0.63
Arnautka	3.70	0.72	0.72	0.13	0	82	0.00	0.59
Stewart	4.92	0.89	0.43	0.15	52	83	0.46	0.74
Kubanka	4.14	1.13	0.55	0.24	51	79	0.58	0.89
Kahla	4.91	1.14	0.75	0.22	34	81	0.39	0.92
Pelissier	4.99	1.37	0.78	0.45	43	67	0.59	0.92
Acme	2.86	0.66	0.36	0.06	45	91	0.30	0.60
Monad	3.32	0.77	0.46	0.00	40	100	0.31	0.77
Pentad	3.29	0.84	0.55	0.21	35	75	0.29	0.63
Bald Medeah	4.17	1.12	0.96	0.43	16	61	0.18	0.69
Golden Ball	4.51	1.71	1.54	0.91	10	47	0.17	0.80

With these data, certain relationships between each phase of the reaction and the various factors have been examined and are discussed below.

The Initial Reaction. A plot of the initial pigment concentration against the rate of the initial reaction indicated that these factors are largely independent; for eight of the thirteen varieties the rates were approximately the same, while for the remaining five the rates were somewhat higher but did not reflect the initial pigment concentration. The rate in cyanide followed the rate in water to some extent, the correlation coefficient being significant beyond the 1% point; however, this was due almost entirely to the effect of the same five varieties which showed a higher rate in water. The rate in alpha naphthol also followed the rate in water, the correlation being highly significant and again, due largely to the same five varieties.

Comparison of the percentage inhibition of the initial reaction by cyanide and alpha naphthol clearly illustrates the difference between the action of these two inhibiting agents; cyanide inhibition varies from 0 to 52% (average value 35%) while alpha naphthol inhibition varies from 47 to 100% (average value 82%). It was suggested in the previous paper (3) that cyanide acted on an activating system rather than directly on the enzyme; this would reduce the effectiveness of the enzymic oxidation but should not reduce it to zero. Alpha naphthol, on the other hand, acts as an antioxidant and thus should largely prevent oxidation of the pigment until the added alpha naphthol is exhausted. This difference is shown more clearly by comparing the pigment equivalents of the alpha naphthol and cyanide inhibition, these vary from 0.59 to 0.92 p.p.m. (mean, 0.76 p.p.m.) for alpha naphthol, and from 0.00 to 0.59 p.p.m. (mean, 0.32 p.p.m.) for cyanide. Although the rate in water varies threefold, from 0.63 to 1.71 p.p.m. per minute, the pigment equivalent of the alpha naphthol inhibition is roughly constant about the mean value of 0.76 p.p.m. In contrast, a plot of the cyanide equivalent against the rate in water shows a wide random scatter with no apparent trend.

The Mixing Reaction. A plot of the initial pigment concentration against the rate of the mixing reaction indicated that there is no relation between these two factors. The rate of the mixing reaction followed the rate of the initial reaction reasonably well; the correlation between these two was 0.87 (1% = 0.68). This would be expected on the basis of the hypothesis proposed for the mechanism of the reaction (3) if all samples had been milled and stored under similar conditions.

For ten of the thirteen samples, the rate in cyanide followed the rate in water very closely; two varieties, Golden Ball and Stewart,

appeared to be more sensitive to cyanide, while Kubanka was less so. These differences did not appear during the initial reaction.

The rate in alpha naphthol appears to be largely independent of the rate in water.

Inhibition by cyanide is more pronounced during the mixing reaction than during the initial reaction, varying from 20 to 100% (average 52%). This behavior contrasts with that of alpha naphthol which affects the mixing reaction much less than the initial reaction, inhibition varying from 59% to three cases of slight acceleration (average 14%); it appears that the amount of alpha naphthol added just prevents the initial reaction but is exhausted early in the mixing reaction. This

TABLE IV

INHIBITION OF MIXING REACTION BY 0.01 *N* CYANIDE AND BY 0.001 *M* ALPHA NAPHTHOL

Variety	Rate of mixing reaction (p.p.m. per minute)			Inhibition %		Overall inhibition %		Activity coefficient
	Water	Cyanide	α-naphthol	Cyanide	α-naphthol	Cyanide	α-naphthol	
Carleton	0.17	0.07	0.17	59	0	44	66	0.13
Mindum	0.17	0.06	0.15	65	12	58	69	0.14
Goose	0.18	0.09	0.10	50	49	45	76	0.15
Arnautka	0.16	0.06	0.16	63	0	26	50	0.16
Stewart	0.27	0.00	0.11	100	59	74	73	0.20
Kubanka	0.20	0.16	0.25	20	*	40	42	0.20
Kahla	0.27	0.18	0.37	33	*	35	33	0.22
Pelissier	0.31	0.20	0.25	35	19	39	48	0.26
Acme	0.20	0.09	0.18	55	10	50	53	0.27
Monad	0.24	0.14	0.27	42	*	40	45	0.29
Pentad	0.23	0.12	0.24	48	0	41	39	0.29
Bald Medeah	0.31	0.20	0.24	36	23	24	44	0.31
Golden Ball	0.44	0.16	0.39	64	11	34	31	0.48

* Slight acceleration.

contrast provides additional support for the hypothesis suggested to account for the behavior of these inhibiting agents.

From the data on overall inhibition, it is clear that *in general* cyanide has a marked inhibiting effect on the reaction responsible for pigment destruction and that the same is true of alpha naphthol.

The Activity Coefficient. The activity coefficient (last column of Table IV) is obtained by dividing the pigment destroyed in the interval from one to four minutes of the mixing reaction by the amount remaining at the end of the initial reaction (after one minute). It appears to be a useful measure of the potential contribution of the yellow pigment factor to macaroni quality. No suggestion is made that this figure gives a reliable index of macaroni-making quality since it takes no account of the brown pigment, and therefore, gives a reasonable meas-

ure of relative macaroni-making quality only when the amount of brown pigment is small. The claim for the activity coefficient is that it indicates relatively how much pigment will remain after a given treatment, and provides a useful index of enzymic activity.

In spite of the large differences in initial pigment content among the varieties studied, the coefficient gives an excellent indication of the enzymic activity as well as a reasonable indication of the amount of pigment remaining after four minutes of mixing. The correlation between activity coefficient and pigment content after four minutes mixing, is 0.80 (1% = 0.68). For this series, then, in which environmental effects were largely eliminated, the activity coefficient is characteristic of the varieties and could be very useful in distinguishing between good and bad varieties. It is interesting to compare these activity coefficients with others calculated for different samples of some of these varieties during the course of this whole investigation:

Pelissier	0.23	Carleton	0.13
(variation 22%)	0.28	(variation 8%)	0.13
	0.26		0.14
Golden Ball	0.49	Mindum	0.27
(variation 18%)	0.42	(variation 240%)	0.34
	0.48		0.10
			0.14

These data emphasize a point which has been noted for many years in testing plant breeders' varieties: Mindum is a rather unstable variety, being as good as the best varieties in some years, but being considerably poorer in others. Although no similar data on the variation of the activity coefficient are available for Stewart, experience with other varietal series has indicated that this variety is also more unstable than Carleton, although not to as marked a degree as Mindum. It is also interesting to note the effect of mixing good and poor varieties. Although the data at present are very limited, it has been found that a mixture of about 50% Golden Ball with 50% of the best varieties yields semolina which has an activity coefficient of 0.35 and gives a reaction curve typical of Golden Ball alone. A more extensive investigation of this phenomenon is planned for the near future.

Literature Cited

1. American Association of Cereal Chemists. Cereal Laboratory Methods. (5th Ed.) Am. Assoc. Cereal Chemists: St. Paul, Minn. (1947).
2. Cunningham, R. L., and Anderson, J. Ansel. Micro tests of alimentary pastes. I. Apparatus and method. Cereal Chem. 20: 171–185 (1943).
3. Irvine, G. N., and Winkler, C. A. Factors affecting the color of macaroni. II. Kinetic studies of pigment destruction during mixing. Cereal Chem. 27: 205–218 (1950).

STUDIES WITH DOGS FED FLOUR TREATED WITH AMMONIUM PERSULFATE[1]

AARON ARNOLD and FRANS C. GOBLE

ABSTRACT

Dogs were fed diets which contained high levels of untreated flour or of flour treated with 0.15% or 1.0% ammonium persulfate for periods of time up to 16 months. Observations were made throughout of gross appearance, weight gains, kidney dye clearances, blood picture (hemoglobin, total red and white cell counts, and differential white cell count including the polymorphonuclear neutrophiles, eosinophiles, lymphocytes, and monocytes), followed by examination of the thoracic and abdominal viscera, the endocrines, the central nervous system at autopsy, and microscopic study of the organs. On the basis of these studies it is concluded that ammonium persulfate, even in amounts which are more than 200 times those which would be present in commercially treated flour, is an innocuous ingredient in the diet.

As a result of Mellanby's report (8) on the toxicity of nitrogen trichloride-treated flour, great interest has been focused on possible undesirable effects of other agents used for treating flour. As indicated in a recent review (1), several investigators have shown that only nitrogen trichloride, of all agents thus far tested in short-term feeding trials, exerts any deleterious action.

This communication gives the results obtained on dogs when ammonium persulfate was used. Reports (6) indicate that this agent is used widely in Europe and at one time most of the flour in England was treated with it. Tests with dogs are practically mandatory in view of the fact that observations with this species first brought to light an unfavorable effect due to the use of nitrogen trichloride for flour treatment. Also, dogs have proven to be the species most sensitive to the effects of the toxic agent produced by the nitrogen trichloride treatment of flour.

Materials and Methods

Diets. The composition of the diets, patterned after those of Mellanby (8), was given in detail in a previous paper (2). In addition to two dogs fed control diets, six dogs were fed a diet which contained flour with 0.15% or 68 g. of ammonium persulfate per cwt (45.4 kg.), and four dogs were fed a diet which contained flour with 1.0% or 454 g. of ammonium persulfate per cwt (45.4 kg.).

[1] Manuscript received February 23, 1950.
Contribution from Sterling-Winthrop Research Institute, Rensselaer, N. Y.

The flour and yeast components of the diet were steamed 90 minutes before feeding. Thus, the persulfate exerted its action upon the flour prior to its ingestion. As observed by Kent-Jones and Amos (6), tests (4) indicated no free persulfate at the end of the steaming period. The end product of persulfate appears to be ammonium sulfate, an agent routinely added to bread doughs as a yeast food. Diets containing nitrogen trichloride-treated flour which had been subjected to steaming in the same way were manifestly toxic to dogs (8).

Dogs. In all, 12 dogs were used in this study. Seven of them had previously not been used for any tests. However, the tests which are given below on five of them, numbers 9, 10, 12, 15, and 17, represent further study on dogs earlier used and reported (2). In that study, the dogs had been subjected to several successive short-term feeding trials of diets which contained one or another of several flour maturing agents. Thus, five of the dogs had experienced serious untoward reactions due to the ingestion of nitrogen trichloride treated flour prior to their use in this portion of the study.

Organ Function Tests. To provide additional and more refined methods of confirming the apparent well-being of the dogs, several tests, such as are used for humans in clinics, were performed at approximately monthly intervals on the dogs.

The test (9) for kidney function was based on measuring the capacity of the kidney to clear phenolsulfonphthalein from the bloodstream. The dye was injected intramuscularly into the dogs and the amount present in urine samples taken by catheterization at ½ and 1 hour after injection was estimated colorimetrically and compared with the amounts injected. Ten readings were obtained on the dogs carried through the entire experimental period.

Supplementary to this, six observations were made during the latter 12 months of test on the amount of urinary protein precipitable by phosphotungstic acid (10) as another measure of possible renal damage. In general, only minute amounts of protein, 0.2g per l of urine, are consistently expected.

Hematological Tests. The 17 hematological examinations of the dogs performed at approximately monthly intervals in all cases comprised the following: Total red and white cell counts, hemoglobin content, and differential white cell count. The normal values for each of the above items are known from numerous studies reported in the literature, but collateral studies on control dogs in our own laboratories have been made throughout the course of these investigations.

Post-mortem Examinations. Five of the dogs were sacrificed by rapid intravenous injection of Evipal Sodium rather early in the tests (after 22 weeks of test for four of the dogs and six weeks of test for the

control dog). The remaining dogs were sacrificed at the termination of the studies after approximately 16 months of test. A gross examination of the thoracic and abdominal viscera, the endocrines, and the central nervous system was made. Material from the various organs was fixed in 10% formalin for subsequent histological examination. Paraffin sections of the following organs, stained with hemalum and eosin, were examined microscopically: heart, lungs, liver, spleen,

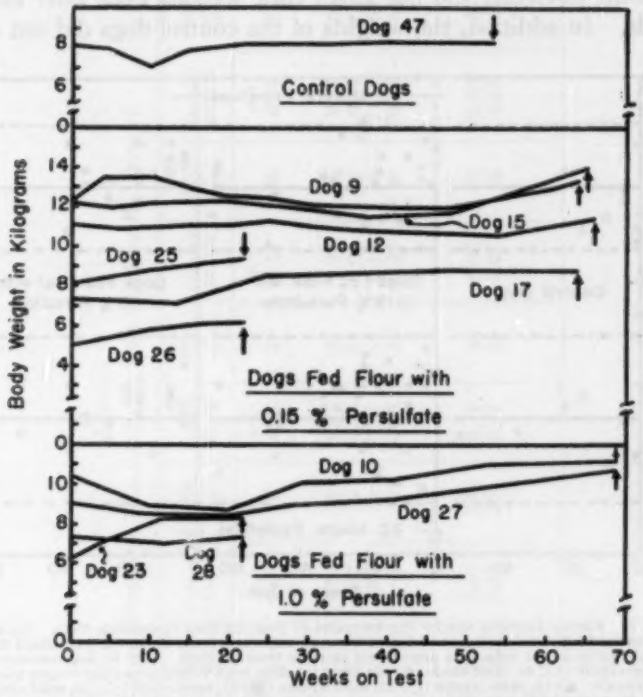

Fig. 1. Weight records of dogs fed diets containing flour treated with none, 0.15%, or 1.0% ammonium persulfate. The arrows indicate the times at which the dogs were sacrificed. Dogs 47, 25, 26, 23, 27 and 28 were fed the indicated diets without any premedication control period. Dogs 9, 10, 12, 15, and 17 were fed the indicated diets subsequent to a 90 day feeding trial of essentially the same regimen except that the flour had been treated with 0.033% ammonium persulfate (2).

kidneys, pancreas, stomach, intestine, lymph nodes, thyroid, adrenal, pituitary, brain (including cerebral cortex, basal ganglia, hippocampus, mammillary bodies, cerebellum, pons), spinal cord, and gonads. In addition, sections of the central nervous system were stained by the following methods for the demonstration of particular structures: Mallory's (7) phosphotungstic acid hematoxylin for glia fibers, cresyl violet for Nissl bodies, Bodian's protargol for axis cylinders and Weil's (11) method for myelin sheaths.

Results

Weight Records. It will be noted from Fig. 1 that the dogs were fed the test diets for 16 months. For five of the dogs, this portion of the study was subsequent to the part reported earlier (2) so that, in all, these five were on test for two years. While a diet high in flour is not particularly well suited to dogs, it is apparent that they were maintained satisfactorily on it. The presence or absence of persulfate treatment obviously did not affect their weights even after extended periods. In addition, the weights of the control dogs did not appear

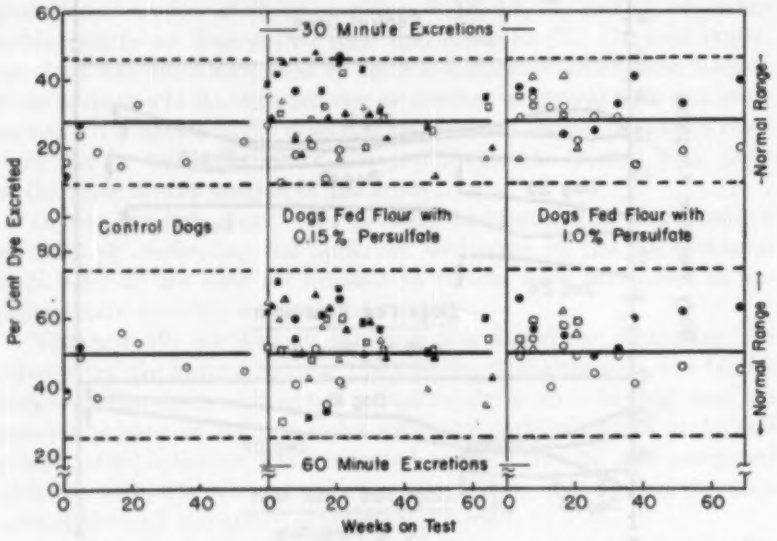

Fig. 2. Kidney function test by dye excretion of dogs fed flour containing diets. Solid lines are average values for normal animals and dotted lines are upper and lower limits (± 2 standard deviations) of normal values in our colony as determined in more than 50 dogs. Key to dog numbers: Control dogs—open circle (○) 47, solid circle (●) 48; Dogs fed flour with 0.15% persulfate—open triangle (△) 9, solid triangle (▲) 12, open square (□) 15, solid square (■) 17, open circle (○) 25, solid circle (●) 26; Dogs fed flour with 1.0% persulfate—open circle (○) 10, open triangle (△) 23, solid circle (●) 27, open square (□) 28.

to differ materially from the test dogs, further indicating a lack of difference in the nutritive value of the diets.

Kidney Function Evaluated by Dye Excretion. The means ± 2 s.d. of the kidney function results observed on over 50 normal dogs in our colony have been indicated for reference. The mean excretion at 30 minutes was 28 ± 9.2% and, at 60 minutes 50 ± 12.2% (means ± s.d.).

From the data given in Fig. 2 it may be seen that the excretions fall within the normal range as here defined. The two high ½ hour values observed early in the test on two of the dogs cannot be regarded

TABLE I

KIDNEY FUNCTION EVALUATED BY URINARY PROTEIN OUTPUT

Dietary persulfate level	Dog no.	Week of test	Urinary protein	Week of test	Urinary protein	Week fo test	Urinary protein	Week of test	Urinary protein	Week of test	Urinary protein	Week of test	Urinary protein	Urinary protein, mean ±s.e.
%			g./l.		g./l.		g./l.		g./l.		g./l.		g./l.	g./l.
None	47	5	Trace	10	0.07	17	0.22	22	0.14	36	0.50	53	0.07	0.17±0.07
None	48	5	0.07											
0.15	9	16	0.07	21	0.14	28	0.22	33	0.22	48	0.07	64	0.65	0.23±0.09
0.15	12	18	0.36	23	0.07	30	0.14	35	0.07	50	0.14	66	0.07	0.14±0.04
0.15	15	17	0.14	22	0.07	29	0.07	34	0.14	49	Trace	65	0.04	0.08±0.02
0.15	17	16	0.07	21	0.43	28	0.36	33	0.22	48	0.23	64	1.20	0.43±0.16
0.15	25	21	0.07											
0.15	26	21	0.07											
1.0	10	21	0.22	26	0.07	33	0.14	38	0.07	52	0.04	69	0.04	0.10±0.03
1.0	27	21	—	26	0.07	33	0.07	38	0.07	52	0.04	69	0.86	0.22±0.16
1.0	23	21	0.07											
1.0	28	21	0.07											

TABLE II

SUMMARY (MEANS ± S.E.) OF HEMATOLOGICAL OBSERVATIONS ON THE DOGS FED DIETS WHICH SUPPLIED EITHER UNTREATED FLOUR OR FLOUR TREATED WITH 0.15% OR 1.0% OF AMMONIUM PERSULFATE

Dietary persulfate level	Dog no.	No. readings	Hemoglobin	Red blood cells $\times 10^6$	White blood cells $\times 10^3$	Neutrophiles $\times 10^3$	Eosinophiles $\times 10^3$	Lymphocytes $\times 10^3$	Monocytes $\times 10^3$
%			mg. %						
None	47	12	13.2 ±.3	6.7 ±.4	20.4 ±1.3	14.2 ±1.4	1.3 ±.2	3.9 ±.4	0.5 ±.1
"	31[1]	4	13.7 ±4.5	6.7 ±.3	10.8 ±1.0	7.0 ±.3	0.6 ±.1	2.8 ±.2	0.3 ±.1
"	32[1]	4	14.8 ±2.2	6.1 ±.2	10.9 ±1.9	8.[illegible] ±1.[illegible]	.[illegible] ±.2	2.0 ±.4	.2 ±.1
"	36[1]	4	17.8 ±.2	7.3 ±.1	11.2 ±1.5	7.5 ±.9	0.5 ±.1	2.8 ±.7	0.4 ±.1
"	38[1]	4	14.8 ±.2	5.9 ±.1	19.5 ±3.5	13.0 ±3.3	1.1 ±.3	4.6 ±.8	0.7 ±.2
"	40[1]	4	16.4 ±.4	6.2 ±.1	15.2 ±2.7	8.0 ±1.2	2.8 ±.7	4.0 ±.9	.5 ±.1
"	43[1]	4	18.7 ±.6	[illegible].8 ±.1	10.1 ±1.5	7.2 ±1.3	0.3 ±.1	1.9 ±.2	0.6 ±.2
0.15	9	17	16.0 ±.3	6.8 ±.2	13.0 ±1.1	6.9 ±.8	1.2 ±.2	4.6 ±.4	0.3 ±.1
"	12	17	14.8 ±.3	6.9 ±.2	17.7 ±1.1	11.1 ±.8	1.0 ±.1	5.2 ±.4	0.4 ±.1
"	15	17	16.0 ±.4	7.1 ±.3	11.6 ±1.1	6.2 ±.8	1.5 ±.2	3.7 ±.3	0.4 ±.1
"	17	17	15.4 ±.2	8.0 ±.3	14.1 ±.7	8.6 ±.6	1.3 ±.5	5.1 ±.4	0.5 ±.1
"	25	5	13.3 ±.4	5.4 ±.3	15.2 ±2.8	9.1 ±2.4	0.7 ±.1	5.1 ±1.0	0.3 ±.1
"	26	5	14.3 ±.2	6.7 ±.4	11.3 ±2.2	8.0 ±1.0	0.6 ±.1	2.3 ±.3	0.4 ±.1
1.0	10	17	16.1 ±.2	7.3 ±.3	12.7 ±.7	8.4 ±.5	0.7 ±.1	3.4 ±.2	0.3 ±.1
"	23	5	16.3 ±.5	6.3 ±.5	13.5 ±2.0	8.7 ±1.5	.6 ±.2	3.8 ±.8	.2 ±.1
"	27	17	17.4 ±.3	7.5 ±.3	12.3 ±.7	8.2 ±.5	0.4 ±.2	3.3 ±.2	0.5 ±.1
"	28	5	14.5 ±.8	6.2 ±.3	12.0 ±2.3	9.5 ±1.8	0.1 ±.1	2.4 ±.6	0.2 ±.1

[1] Hematological readings on six control dogs which were not otherwise associated with this study.

as significant evidence of poor function in view of the normal values on the same dogs subsequently.

Kidney Function Evaluated by Urinary Protein. The data on the urinary protein excretions are given in Table I. It may be seen that there are no consistent trends of progressive increase of urinary protein output and that the means of the three groups overlap so that no evidence of kidney derangement can be ascribed to any of the dietary treatments. Whether catheterization of the dogs in taking the samples contributed to the wide range of values obtained cannot be indicated with certainty, though it is believed that this may have influenced the results to some extent.

Urinary Calculi. The control urine samples were examined for sediment. The samples were by no means free of casts, bacterial cells, "triple phosphates", etc. but these were not present to a marked extent in any of the dogs, nor were any of the three groups of dogs distinguished by any differences. Also, consistent progressive increases in urinary sediment in the dogs could not be established. Here again, irritation due to catheterization probably affected the findings.

Hematological Examinations. The hematological findings were calculated so as to yield an individual value for each dog (mean $\pm$ s.e.). For added comparative purposes, the readings on six control dogs, in no way connected with these studies, have been included in Table II.

It may be noted that, in the elements given in the table, there are no trends which can be correlated with the ingestion of ammonium persulfate treated flour. There is as much variation among the dogs within a group as among the dogs between groups. Thus, this method of following the well-being of the animals disclosed no adverse findings.

Post-mortem Examinations. Neither gross nor microscopic examination of the organs of the dogs on test revealed any tissue changes attributable to the ingestion of the ration which included ammonium persulfate treated flour. No conditions were found which did not also occur in the control dogs fed untreated flour. Parenthetically, it may be noted that a careful study did not disclose any lesions in sections of the tissues of two dogs sacrificed while showing symptoms of running fits induced by nitrogen trichloride treated flour.

Discussion

Under conditions of test which were far more rigorous than those which lead to running fits in dogs fed nitrogen trichloride treated flour, dogs fed ammonium persulfate treated flour show no ill effects. Thus, dogs fed nitrogen trichloride treated flour at commercial levels of treatment (8) developed running fits in a matter of two to four weeks. In contrast, dogs fed ammonium persulfate treated flour for periods up

to 16 months at levels which were approximately 200 times the level of treatment required to achieve maturing action evidenced no adverse reaction as judged by weight maintenance, kidney dye clearance, urinary protein outputs, hematological examinations, post-mortem examinations, or by tissue section study. These observations are in agreement with our own earlier preliminary studies (2) and those of Bentley *et al.* (5).

These observations are in accord, also, with results on rats fed flour containing ammonium persulfate and bread baked from flour treated with ammonium persulfate (3). And finally, this agent has been used extensively on flour intended for human consumption in Europe as indicated by Kent-Jones and Amos (6).

On the basis of the studies summarized here we may conclude that ammonium persulfate is an innocuous ingredient when used for the maturing of flour.

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Literature Cited

1. Arnold, A. Current status of flour maturing agents from the biological standpoint. Trans. Am. Assoc. Cereal Chem. **7**: 73–77 (1949).
2. Arnold, A. Effect on dogs of flours treated with various improving agents. Cereal Chem. **26**: 46–51 (1949).
3. Arnold, A., and Goble, F. C. Ammonium persulfate as a flour maturing agent. Fed. Proc. **8**: 377 (1949).
4. Auerbach, M. E., Eckert, H. W., and Angell, E. Determination of persulfate in flour and dough. Cereal Chem. **26**: 490–496 (1949).
5. Bentley, H. R., Booth, R. G., Greer, E. N., Heathcote, J. G., Hutchinson, J. B., and Moran, T. Action of nitrogen trichloride on proteins. Production of toxic derivatives. Nature **161**: 126–7 (1948).
6. Kent-Jones, D. W., and Amos, A. J. Modern cereal chemistry. The Northern Publishing Co., Ltd., Liverpool (1947).
7. Mallory, F. B. Pathological technique. W. B. Saunders Co., Philadelphia (1938).
8. Mellanby, E. Diet and canine hysteria. Brit. Med. J. **2**: 885–887 (1946).
9. Rowntree, L. G., and Geraghty, J. T. An experimental and clinical study of the functional activity of the kidney by means of phenolsulphonephthalein. J. Pharmacol. Exptl. Therap. **1**: 579–661 (1909).
10. Shevsky, M. A., and Stafford, D. D. A clinical method for the estimation of protein in urine and other body fluids. Archiv. Internal Med. **32**: 222–225 (1923).
11. Weil, A. Textbook of neuropathology. Grune and Stratton, New York (1945).

BREAD LOAF VOLUME AND PROTEIN CONTENT OF HARD RED SPRING WHEATS [1]

C. C. FIFIELD,[2] RAY WEAVER,[3] AND J. F. HAYES [3]

ABSTRACT

The relation between loaf volume and flour protein is reported for 589 samples representing ten varieties of hard red spring wheat grown for four crop years under a wide range of climate and soil conditions. A rich formula containing milk solids, shortening, and potassium bromate in conjunction with optimum mixing time and water requirement was employed in the bread-baking tests.

The relation between loaf volume and flour protein for each variety was linear between the limits of protein encountered, approximately 8.5 to 18.0%. The regression lines for loaf volume on protein content for any variety were similar for the years 1944, 1945, 1946, and 1947, indicating that the bread-baking quality of each variety was essentially the same in different years.

The level and slope of the regression of loaf volume on protein content for the varieties differed significantly, indicating differences between varieties in protein quality.

It is well known that early investigations of the relationship between protein content of wheat and bread loaf volume by Thomas (12), Shollenberger (11) and others indicated a curvilinear relation. Bailey and Sherwood (1) calculated the formula for the curve based on the data of five crop years, 1921–1925.

Zinn (13) in a study of the relationship of the different characters of American grown wheats based on published data (1907 to 1919) obtained high correlation coefficients between loaf volume and protein content. Larmour (8) found with samples of Canadian hard red spring wheats from the 1929 crop that the regression of loaf volume on protein was linear between the limits of 7.0% and 19.3% when a blend-bromate formula was used. Using baking techniques that more fully reflect the potentialities of each flour, linear relations within varieties have since been demonstrated by Barmore, Finney and McCluggage (2, 3) Larmour, Working and Ofelt (9), and Bayfield and West (4). Barmore, Finney and McCluggage (3) and McCalla (10) showed that the level and slope of the regression lines are different for different varieties. Exceptions to this linear relation may occur as a result of

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deleterious effect of excessive high temperature between heading and ripening of the grain on loaf volumes, as shown by Finney and Fryer (7).

If it can be shown that the relation between protein content and loaf volume is generally linear for all classes of wheat, the characterization and evaluation of varieties will be greatly facilitated. This will be true especially for samples that differ in protein content because of inherent differences or environmental conditions. The purpose of this paper is to present additional data bearing on the relation between protein content and loaf volume in hard red spring wheat varieties.

Materials and Methods

This paper presents results for 589 samples representing ten varieties of hard red spring wheat grown in experimental plots at approximately 25 stations in nine states in the years 1944, 1945, 1946, and 1947. All the samples were grown in the hard red spring wheat region but environmental conditions differed widely between some of the experiment stations. The varieties were Thatcher, Pilot, Rival, Regent, Mida, Ceres, Cadet, Newthatch, Henry, and Pilot × Mida No. 1756 (C. I. No. 12303). The number of samples of each variety tested each year varied from six for the variety Henry to 25 for Thatcher.

The samples used in this study were grown under variable but, in general, rather favorable seasonal conditions. The 1944 growing season was perhaps the best of the four. Although seedings were late the rainfall was abundant, resulting in good yields. The quality of the grain was good, and protein content was about average. The seasons in 1945 and 1946 were late and cool and except for some stem and leaf rust damage the yields were normal and the grain was of good quality. The protein content was high in both of these years with 1946 averaging higher. Excessive rain and cold weather delayed planting of the 1947 crop. The moisture conditions during the growing season were generally good except in northwestern North Dakota and northern Montana where there was a deficiency in moisture. There appears to have been no damage to quality because of high temperature such as that reported by Finney and Fryer (7). The grain was of generally good quality and averaged about the same in protein content as the samples of the 1944 crop.

The samples were milled to 90 per cent patent flours on the Allis-Chalmers experimental flour mill. The flours ranged from 8.5 to 18.0% in protein content and 0.41 to 0.53% in ash content (14.0% moisture basis). The bread-baking tests were made by a formula described by Fifield, *et al.* (5) using 100 grams of flour, 2.0 grams of compressed yeast, 1.5 grams of salt, 5.0 grams sugar, 0.25 grams of malted wheat flour, 3.0 grams of shortening, 4.0 grams of non-

fat dry milk solids, and varying amounts (0 to 4 mg.) of potassium bromate. The ingredients for two loaves of bread were mixed for a sufficient length of time for optimum dough development in a Hobart-Swanson dough mixer with four pins in the head and two pins in the bowl and operated at 108 r.p.m. The doughs after mixing were divided into two equal parts, fermented for 3 hours at 86°F. (30°C.), panned and proofed for 55 minutes at 86°F. (30°C.) then baked for 25 minutes at 450°F. Loaf volumes and other data were averaged for the two loaves. Loaf volume data are presented only for those loaves containing that amount of potassium bromate that produced maximum loaf volumes. In most instances the loaf having the greatest volume also had the best grain, texture, and crumb color. This procedure is substantially the same as that used by Finney and Barmore (6).

Results and Discussion

Comparison of Interannual Results Within Variety. The number of samples, correlation coefficients, and regression equations for bread loaf volume vs. protein content of flour for each variety in each of the years 1944 to 1947 are given in Table I. The relations between loaf volume and flour protein content are shown graphically by means of regression lines in Fig. 1.

The relation between loaf volume and protein content was essentially linear within each variety, as shown in Fig. 1. It is evident from these coefficients (r varies from + 0.77 to + 0.99), which were all found, to be significant at the 1% level, that protein content accounts for a large part of the variation in loaf volume within a variety regardless of season.

The protein content of the samples of each variety in this study with the exception of Henry, ranged from approximately 8.5 to 18.0% depending upon the environment under which they were grown. The protein content for Henry varied from 9.0 to 14.0%. There were fewer high protein samples of Henry, because it was not grown at the stations in the western part of the region where high protein samples of other varieties are generally produced.

The slopes of the regression lines (Fig. 1) for any individual variety were similar for each of the four years. None were significantly different as shown by covariance analysis. The greatest variation appears to be for the variety Henry between the 1946 and 1947 seasons, probably due to the relatively small number of samples for this variety. The agreement between the regression lines for different years seems to be best for Pilot, Cadet, Newthatch, Regent, and Mida. The data indicate that the increment of increase in loaf volume per unit increase

TABLE I

ANNUAL CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS FOR LOAF VOLUME AND FLOUR PROTEIN FOR 10 VARIETIES OF HARD RED SPRING WHEAT

Variety	Number of samples				Correlation coefficient[1]				Regression equation $\hat{Y} =$			
Year	1944	1945	1946	1947	1944	1945	1946	1947	1944	1945	1946	1947
Newthatch	14	18	19	19	0.88	0.86	0.81	0.80	190+49.8 X	301+40.9 X	243+44.4 X	256+44.7 X
Ceres	6	9	11	9	0.97	0.99	0.90	0.96	116+54.0 X	117+54.4 X	349+39.0 X	195+49.2 X
Cadet	14	18	19	19	0.90	0.91	0.85	0.93	199+52.4 X	301+43.5 X	212+50.0 X	267+45.3 X
Thatcher	18	23	25	24	0.94	0.91	0.86	0.89	3.8+65.4 X	223+48.8 X	246+45.8 X	218+49.3 X
Regent	10	12	14	15	0.97	0.91	0.89	0.87	98+59.4 X	214+50.8 X	132+56.6 X	295+44.1 X
Mida	14	18	21	20	0.95	0.85	0.83	0.77	112+56.6 X	228+47.7 X	215+46.9 X	254+42.9 X
Rival	10	11	14	15	0.94	0.80	0.90	0.87	56+65.5 X	88+59.9 X	16+64.3 X	91+58.8 X
Pilot	14	19	20	20	0.93	0.93	0.85	0.89	208+52.4 X	135+58.8 X	273+46.4 X	167+54.4 X
Pilot × Mida (N1756)	7	13	12	14	0.94	0.95	0.89	0.89	243+45.3 X	245+46.8 X	205+47.8 X	62+58.7 X
Henry	6	6	10	9	0.97	0.87	0.82	0.93	280+47.6 X	441+34.4 X	178+54.2 X	47+66.7 X

[1] All correlation coefficients are significant at the 1% level.

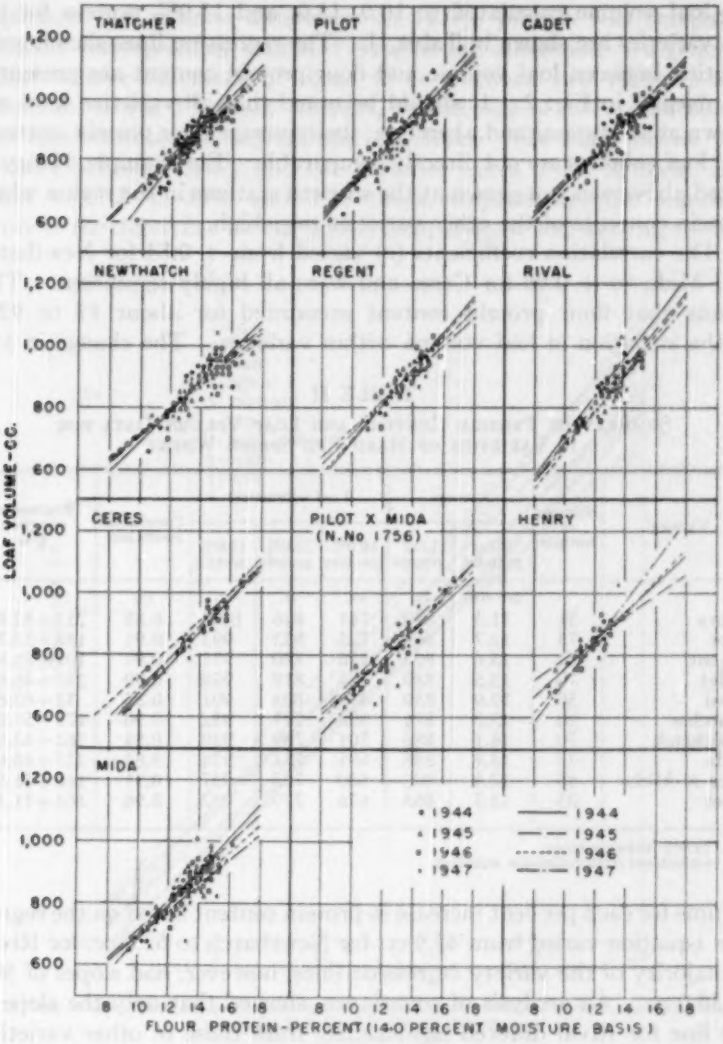

FIG. 1. Annual regression lines for loaf volume and flour protein for ten hard red spring wheat varieties grown for the 1944–1945–1946 and 1947 seasons.

in protein content is essentially the same for any one variety in different years.

Comparison of Varieties. Since the regression lines for the same variety are substantially the same for different seasons, a comparison of varieties based on the average of all seasons seems justified. The four-year average protein content, loaf volume, regression equations, and

the loaf volume calculated to 10.0, 12.0, and 15.0% protein for the ten varieties are shown in Table II. The regression lines showing the relation between loaf volume and flour protein content are presented graphically in Fig. 2. It should be noted that all varieties were not grown at all stations and, therefore, their average flour protein contents and loaf volumes are not directly comparable. For example, Henry as noted above was not grown at the western stations in the region where protein contents of the other varieties were high.

The correlation coefficients (r) varied from + 0.84 for Newthatch and Mida to + 0.96 for Ceres and were all highly significant. This means that flour protein content accounted for about 17 to 92% of the variation in loaf volume within varieties. The change in loaf

TABLE II

SUMMARY OF PROTEIN CONTENT AND LOAF VOLUME DATA FOR 10 VARIETIES OF HARD RED SPRING WHEAT

Variety	Number of samples	Average		Loaf volume at[2]			Correlation coefficient	Regression equation $\hat{Y} =$
		Flour protein[1]	Loaf volume	10.0% protein	12.0% protein	15.0% protein		
		per cent	*cc.*	*cc.*	*cc.*	*cc.*	(*r*)	
Henry	31	11.5	820	741	846	1005	0.88	213+52.8 X
Pilot	73	12.7	869	725	832	993	0.91	188+53.7 X
Regent	51	13.4	892	716	820	975	0.91	197+51.9 X
Cadet	70	13.5	889	725	819	959	0.90	257+46.8 X
Rival	50	12.9	859	678	803	991	0.86	52+62.6 X
Thatcher	90	13.5	874	693	797	952	0.90	175+51.8 X
Newthatch	70	14.1	880	701	789	920	0.84	262+43.9 X
Mida	73	13.1	835	691	784	924	0.84	225+46.6 X
Pilot × Mida	46	12.4	803	680	783	937	0.91	165+51.5 X
Ceres	35	13.7	865	676	779	932	0.96	163+51.3 X

[1] 14.0% moisture basis.
[2] Calculated from regression equation.

volume for each per cent increase in protein content based on the regression equation varied from 43.9 cc. for Newthatch to 62.6 cc. for Rival. A majority of the variety regression lines, however, had slopes of 50.0 to 56.0 cc. An analysis of covariance, showed that only the slope of the line for Rival differed significantly from those of other varieties. It can be observed in Fig. 2 that high protein flours of Rival produced relatively large loaves of bread; whereas, low protein flours of this variety generally made smaller loaves than did flours of Thatcher having comparable protein content.

Significant loaf volume differences exist between certain of the hard spring varieties at the various protein levels. For example, at 12.0% protein the loaf volume for Henry was 846 cc. in contrast to 779 cc. for Ceres and 783 cc. for Pilot × Mida. Each of the more

important loaf volume differences at 12.0% protein was compared with the corresponding standard error of the difference. In general, a loaf volume difference of about 45 cc. between two varieties was significant at the 5% level; whereas, a difference of more than 60 cc. was significant at or beyond the 1% level. The loaf volume differences between Henry and the varieties Ceres, Pilot × Mida, and Mida were 2.91, 3.10, and 3.77 times, respectively, the standard errors of the difference for the paired comparisons, and were significant

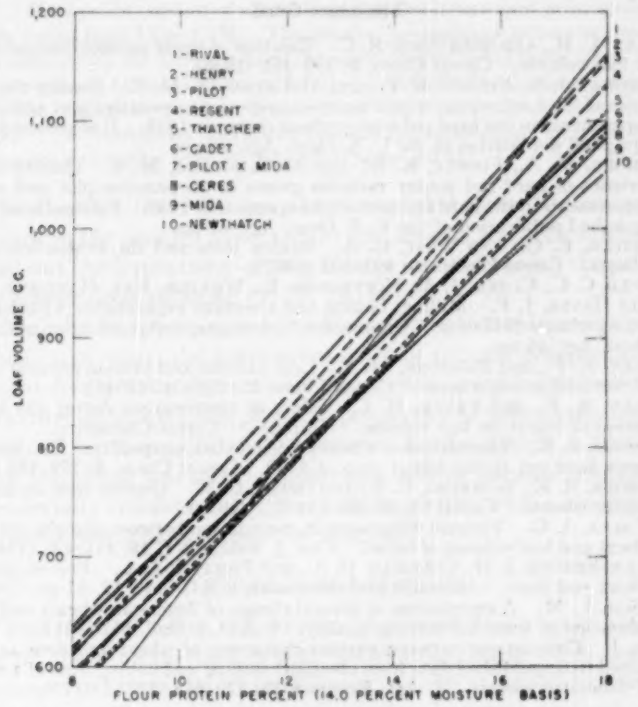

FIG. 2. Loaf volume-flour protein content regression lines for ten varieties of hard red spring wheat grown for four years at several stations.

beyond the 1% level. The average load volume differences of about 50 cc. between Pilot and the varieties Ceres and Pilot × Mida were found to be significant at the 5% level. The difference of 41 cc. between Regent and Ceres approached significance $t = 1.94$. Significance at the 5% level would require that $t = 1.99$.

Studies of this laboratory and of Finney and Barmore (6) indicate that regression lines for loaf volume on protein content are reliable indexes of gluten quality and the potential breadmaking value of vari-

eties of wheat. Loaf volume, of course, depends both on the quality and the quantity of the protein in the flour as well as on certain other variable factors.

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Literature Cited

1. Bailey, C. H., and Sherwood, R. C. Relation of crude protein content of flour to loaf volume. Cereal Chem. **3**: 393–401 (1926).
2. Barmore, M. A., Finney, K. F., and McCluggage, M. E. Quality characteristics of hard red winter wheat varieties grown in cooperative plot and nursery experiments in the hard red winter wheat region in 1938. Unnumbered mimeographed publication of the U. S. Dept. Agr. 26 pp.
3. Barmore, M. A., Finney, K. F., and McCluggage, M. E. Quality characteristics of hard red winter varieties grown in cooperative plot and nursery experiments in the hard red winter wheat region in 1939. Unnumbered mimeographed publication of the U. S. Dept. Agr. 31 pp.
4. Bayfield, E. G., and West, G. A. Baking tests and the evaluation of new wheats. Cereal Chem. **19**: 493–507 (1942).
5. Fifield, C. C., Clark, J. A., Hoffecker, E., Weaver, Ray, Hartsing, T. F., and Hayes, J. F. Milling, baking and chemical experiments with hard red spring wheats 1947 crop. Unnumbered mimeographed publication of the U. S. Dept. Agr. 46 pp.
6. Finney, K. F., and Barmore, M. A. Loaf volume and protein content of hard winter and spring wheats. Cereal Chem. **25**: 291–312 (1948).
7. Finney, K. F., and Fryer, H. C. Effect of temperature during the fruiting period of wheat on loaf volume. (In press.) Cereal Chemistry.
8. Larmour, R. K. The relation of wheat protein to baking quality. II. Saskatchewan hard red spring wheat crop of 1929. Cereal Chem. **8**: 179–189 (1931).
9. Larmour, R. K., Working, E. B., and Ofelt, C. W. Quality tests on hard red winter wheats. Cereal Chem. **16**: 733–752 (1939).
10. McCalla, A. G. Varietal differences in the relation between protein content of wheat and loaf volume of bread. Can. J. Research C **18**: 111–121 (1940).
11. Shollenberger, J. H., Coleman, D. A., and Phillips, C. L. Protein gluten in wheat and flour. Abstracts and references, U.S.G.S.A., 12, 53 pp. (1923).
12. Thomas, L. M. A comparison of several classes of American wheats and a consideration of some influencing quality. U.S.D.A. Bull. 557 (1917).
13. Zinn, J. Correlations between various characters of wheat and flour as determined from published data from chemical, milling and baking tests of a number of American wheats. J. Agr. Research **23**: 529–548 (1923).

THE CYSTINE CONTENT OF WHEAT FLOUR IN RELATION TO DOUGH PROPERTIES [1]

B. WÖSTMANN [2]

ABSTRACT

The cystine content of wheat flour proteins determined polarographically varied from 2.2 to 4.5%. The physical properties of salt-water doughs, as reflected by the surface area of the extensograms for 23 flours taken after a rest-time of 135 minutes are positively correlated with protein content, the cystine content of the flour proteins, and the total cystine content of the flour. The correlation coefficients between the extensogram area and these respective variables were +0.82, +0.77, and +0.92.

The disulfide bond has long been regarded as an important structural unit in the protein molecule (8) and several research workers have pointed out the importance of this linkage in determining the properties of the wheat flour proteins (1, 13, 16, 17).

As part of an investigation of the factors which determine the baking quality of flour, the cystine contents of protein preparations from 23 wheat flours were determined by a polarographic method and the results correlated with the physical properties of salt-water doughs, as determined by means of the Brabender Extensograph. The polarographic technique was used to determine cystine because the results are not materially influenced by the carbohydrates which are present in the protein preparations used for analysis.

Materials and Methods

Wheat Flours. For this study 23 wheat flours were employed; seven of these were hard wheat flours and three were soft wheat flours imported from the United States in 1946 and 1947 by the Dutch government. The remainder were soft flours of 80% extraction milled from Dutch wheat. As far as is known, none was bleached.

Polarographic Determination of Cystine in Flour Protein Hydrolysates. Cystine was determined by a polarographic method based on that described by Brdicka (6) in 1934. He showed that in 0.1 *N* ammonium hydroxide − 0.1 *N* ammonium chloride as supporting electrolyte, cystine gives a characteristic current-voltage curve in the polarograph if 0.001 *N* cobaltous chloride is added. The cystine is reduced to cysteine at −0.4 volts at the dropping mercury electrode and then

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reacts with cobaltous ions to form a complex at the mercury cathode. Starting at −1.5 volts this complex catalyzes an evolution of hydrogen at the cathode interface, the amount of which is dependent upon the concentration of the complex on the cathode. As long as there is a surplus of cobaltous ions with respect to the amount of cystine present, the concentration of the complex on the cathode surface will be determined by the amount of cystine diffusing to the cathode.

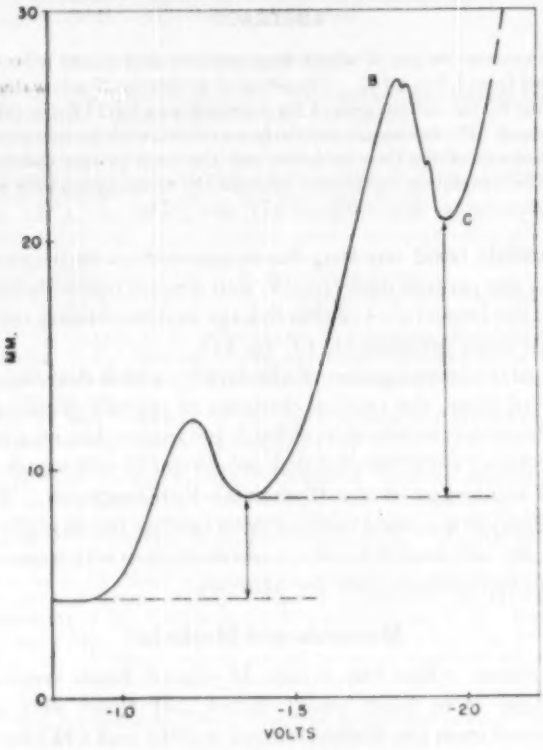

FIG. 1. Polarogram of cystine. At −1.4 volts wave given by Co^{++} ions, at −1.9 volts minimum of cystine wave.

As a result of the catalytic evolution of hydrogen on the cathode interface which starts at a potential of −1.5 volts, the current- voltage curve assumes the configuration shown in Fig. 1. The potentials are measured against the saturated calomel electrode.

Brdicka took the height of the minimum, C, as a measure of the cystine concentration. In later publications, however, he occasionally used the maximum, B, (7, 10). He pointed out that at concentrations between 2×10^{-5} and 10×10^{-5} moles cystine per liter, the minimum

height is not directly proportional to the cystine concentration, the increase in the height being relatively less as the concentration of cystine increases. Moreover, Sladek and Lipschutz (14) showed that the wave heights for cystine were depressed by the presence of other amino acids. For these reasons Stern, Beach, and Macy (15) employed a special calibration technique for determining the cystine content of protein hydrolysates. Increments of a standard cystine solution were

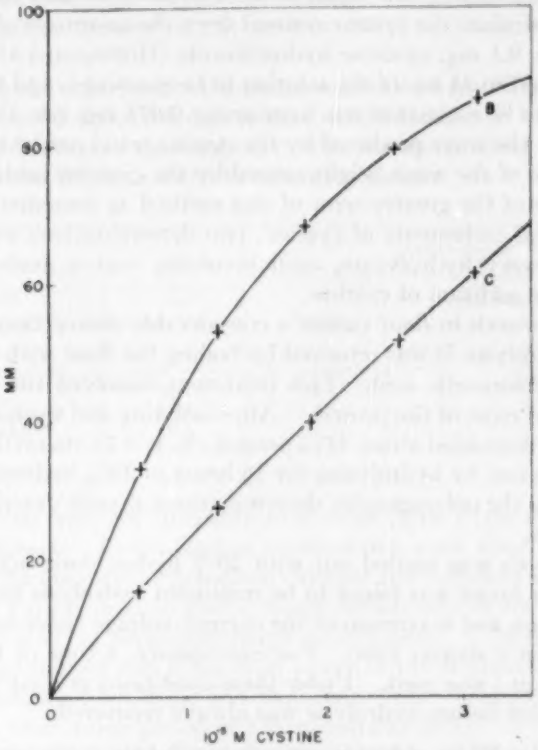

FIG. 2. Height of the maximum B and of the minimum C of the cystine wave as a function of the cystine concentration.

added to a given volume of each hydrolysate under study and the relative changes in the height of the polarographic wave were measured. By this method, calibration curves were obtained which applied to the estimation of cystine in that particular hydrolysate. They reported that this technique permitted determining cystine in solutions containing only 12 to 24 μg/ml. with an accuracy of ±5%.

Using a drop time of one second and an undamped galvanometer period of 2.5 seconds, a somewhat different conclusion was reached in

the present study. As long as the concentration did not rise above 4×10^{-6} moles cystine per liter, the height at the minimum remained almost directly proportional to the concentration as shown in Fig. 2. If different quantities of cystine were added to a protein hydrolysate, this relation was absolutely linear, provided the total concentration remained below the maximum cited above.

For this reason, it was thought justifiable to add an amount of cystine to the hydrolysate equal to the amount estimated to be present and then calculate the cystine content from the minimum of the curve. In practice, 0.1 mg. cysteine hydrochloride (Hoffmann-LaRoche) was always added to 25 ml. of the solution to be examined, and the amount of cystine to be estimated was kept under 0.075 mg. per 25 ml. This meant that the wave produced by the cystine could not be higher than the increase of the wave height caused by the cysteine addition.

Because of the greater error of this method as compared with that using several increments of cystine, two determinations were usually made on every hydrolysate, each involving curves made with and without the addition of cystine.

As the starch in flour causes a considerable destruction of cystine during hydrolysis, it was removed by boiling the flour with a 5% solution of trichloracetic acid. This treatment dissolved the starch and precipitated most of the protein. After isolating and washing the residue, which contained about 45% protein (N × 5.7), its cystine content was determined by hydrolysing for 16 hours in 20% hydrochloric acid, and making the polarographic determinations already described on the hydrolysate.

Hydrolysis was carried out with 20% hydrochloric acid (analar). Six to eight hours was found to be minimum hydrolysis time because the minimum and maximum of the current-voltage curve could not be developed in a shorter time. For convenience, a time of 16 hours (5 p.m. to 9 a.m.) was used. Under these conditions at least 90% of the cystine added before hydrolysis was always recovered.

From 100 to 200 mg. of flour protein containing 1–4 mg. cystine were hydrolyzed in 10 ml. of 20% hydrochloric acid, the humin which formed was removed by filtration and the filtrate was made up to 50 ml. with distilled water.

A current-voltage curve was made on a solution containing 10 ml. 0.2 *N* ammonium hydroxide solution, 10 ml. of a solution containing ammonium chloride (0.2 *N*) and cobalt chloride (0.002 *N*), 1 ml. of protein hydrolysate, and 4 ml. of distilled water. A current-voltage curve was also made by replacing 1 ml. of distilled water in the preceding solution with 1 ml. of a freshly prepared aqueous solution of cysteine hydrochloride (25 mg./250 ml.).

As a check on the method, a recovery experiment was conducted in which cystine was added to a pea flour protein preparation before hydrolysis. Since the cystine content of the protein of pea flour was only about one-fifth of that of wheat flour protein, the proportion of

cystine in relation to the other constituents is much more unfavorable. To 100 mg. samples of the protein precipitated from pea flour by boiling with 5% trichloracetic acid, 0.3 and 0.6 mg. of cystine, respectively, were added and the mixture hydrolized by boiling with 10 ml. of 20% hydrochloric acid solution for 16 hours. After filtration the hydrolysate was brought to a pH of 5 to 6 to permit the addition of more than 1 ml. to the supporting electrolyte without appreciably changing the pH. The protein preparation contained 42.7% protein and the cystine content of the protein (N × 5.7) was found to be 0.76%.

In triplicate experiments the mean recoveries were 98 and 94% for the addition of 0.3 and 0.6 mg. cystine, respectively.

As a further test of the method, cystine was determined in hydrolysates of several proteins with the following results:

Protein	N %	Cystine content		
		Found %	Value from literature	
			%	
Fibrin	16.7	1.0	1.1	(2)
Gliadin	15.4	1.6	2.1	(3)
Globin (man)	14.6	1.2	1.15	(15)
Casein	12.6	0.41	0.37	(4)

The values compare favorably with those given in the literature.

Extensograms. Extensograms representing each flour were made by mixing the flour with 2% sodium chloride and sufficient water in the Brabender Farinograph to give a dough consistency of 500 Brabender units at the point of maximum dough development. The doughs were rounded and formed into cylindrical pieces in the Brabender Extensograph as described by Munz and Brabender (11). The extensograms were made after a rest period of 135 minutes at 30°C.

The surface areas (Cm^2) of the extensograms were measured and the ratio: resistence to extensibility, F (in extensograph units)/extensibility, E (mm.), calculated. This ratio gives an impression of the shape of the extensogram (11).

Results and Discussion

The results of the cystine determinations and extensograms for the wheat flours are summarized in Table I. The percentage cystine in wheat flour proteins reported in the literature (5) range between 1.8 to 2.1%[1] so that the values of 2.2 to 4.5% found in the present study

[1] Calculated for protein = N × 5.7.

are considerably higher. This may be due to compounds of the thiazolidine carboxylic acid type being estimated as free cystine by the polarographic method. These compounds could result from the condensation of cystine with carbohydrate degradation products (12). However, when using a modified Folin-method (phospho-18-tungstic acid reagent) Gubler and Greaves (9) found 0.34–0.41% cystine in the

TABLE I

THE RELATION BETWEEN THE PROTEIN AND CYSTINE CONTENTS OF WHEAT FLOURS AND DOUGH PROPERTIES, AS MEASURED BY THE BRABENDER EXTENSOGRAPH

Sample no.	Protein (N × 5.7) %	Cystine content		Extensogram characteristics[1]	
		Protein %	Flour %	Area Cm.²	*F/E*
AMERICAN FLOURS					
1	11.7	4.0	0.47	148	6.4
2	11.2	4.1	0.46	100	5.8
3	11.1	3.8	0.43	102	—
4	11.1	4.1	0.45	135	3.1
5	10.5	4.5	0.47	129	7.5
6	10.4	4.2	0.44	81	3.8
7	8.1	3.5	0.28	19	1.1
8	7.3	3.5	0.26	49	5.5
9	7.2	3.0	0.22	31	3.2
10	7.1	3.6	0.26	30	2.3
DUTCH FLOURS					
11	9.6	2.2	0.21	28	0.8
12	9.4	4.1	0.38	68	2.7
13	9.1	2.5	0.23	27	1.2
14	9.1	3.9	0.34	70	4.2
15	8.9	3.2	0.29	14	0.4
16	8.8	2.9	0.26	29	1.0
17	8.8	3.0	0.26	21	0.5
18	8.7	4.5	0.39	68	4.7
19	8.7	2.8	0.24	29	1.6
20	8.7	3.0	0.26	20	0.6
21	8.4	3.0	0.25	31	2.0
22	8.3	3.0	0.25	19	0.4
23	8.3	3.0	0.25	20	0.5

[1] The extensograms were made after a rest period of 135 minutes. *F* represents the ratio of the resistance to extension in Brabender units and *E* the extensibility in Cm.

grain. This indicates about 3.5% for flour protein, which gives better agreement with the present results.

The flour samples in Table I are arranged in order of protein content and it is readily seen, as is well known, that the area of the extensogram decreases with a decrease in protein content. The correlation coefficient between the data for these variables is +0.82. Similarly, the cor-

relation between the surface area of the extensogram and the cystine percentage of the protein is +0.77.

These results indicate that the extensogram area is a function of both the quantity of protein present and of the cystine content of the protein. If these variables are combined to give the cystine contents of the flour, an even better correlation with the surface area of the extensogram is obtained; namely, $r = +0.92$.

It may be concluded that the quality of flour protein for bread-making purposes increases with an increase in the number of possible –S–S– linkages, as determined by its cystine content. The total cystine, that is the total number of possible –S–S– linkages, largely determines those properties of flour which are measured by the extensogram.

Literature Cited

1. Balls, A. K., and Hale, W. S. Further studies on the activity of proteinase in flour. Cereal Chem. **13**: 656–664 (1936).
2. Block, R. J., and Bolling, Diana. The amino acid composition of proteins and foods. Charles C. Thomas, Springfield, Illinois (1945).
3. *Ibid.*
4. *Ibid.*
5. *Ibid.*
6. Brdicka, R. Polarographische Mikrobestimmung von Cystin und Cystein in Hydrolysaten von einigen Proteinen. Mikrochemie **15**: 167–180 (1934).
7. Brdicka, R. Wielands labiler Wasserstoff bei der schwermetallkatalytischen Oxydation von Sulfhydrylverbindungen. Biochem. Z. **272**: 104–112 (1934).
8. Crammer, J. L., and Neuberger, A. The state of tyrosine in egg albumen and in insulin as determinated by spectrophotometric titration. Biochem. J. **37**: 302–310 (1943).
9. Gubler, C. J., and Greaves, J. E. Cystine content of wheat and its quantitative determination. Food Research **7**: 405–413 (1942).
10. Kolthoff, I. M., and Lingane, J. J. Polarography. Interscience, New York (1941).
11. Munz, E., and Brabender, C. W. Prediction of baking value from measurements of plasticity and extensibility of dough. I. Influence of mixing and molding treatments upon physical dough properties of typical American wheat varieties. Cereal Chem. **17**: 78–100 (1940).
12. Ratner, Sarah, and Clarke, H. T. The action of formaldehyde upon cysteine. J. Am. Chem. Soc. **59**: 200–206 (1937).
13. Ritter, K. The structure of the proteins and the protein constituents of wheat flour. Mühlenlab. **9**: 1–12; 25–28 (1939).
14. Sladek, J., and Lipschütz, M. Polarographic studies with the dropping mercury Kathode. Part XLIII. Specific effects of some amino acids. Coll. Czechoslov. Chem. Commun. **6**: 487–497 (1934).
15. Stern, A., Beach, E. F., and Macy, I. G. Polarographic microdetermination of cystine in protein hydrolysates. J. Biol. Chem. **130**: 733–740 (1939).
16. Sullivan, Betty, Howe, Marjorie, and Schmalz, F. D. On the presence of glutathione in wheat germ. Cereal Chem. **13**: 665–669 (1936).
17. Sullivan, Betty, Howe, Marjorie, Schmalz, F. D., and Astleford, G. R. The action of oxidizing and reducing agents of flour. Cereal Chem. **17**: 507–528 (1940).

A METHOD FOR THE DETERMINATION OF LACTOSE IN BREAD[1]

R. P. Choi, C. W. Tatter, and C. M. O'Malley

ABSTRACT

A simple fermentation method has been developed for the determination of lactose and indirectly nonfat milk solids in bread. The procedure involves extracting the sugars from bread with dilute alcohol, destroying the non-lactose reducing sugars by fermenting the alcohol-free extract for 2½ hours at 30°C. with baker's yeast, and analyzing for lactose by means of the Somogyi micro-method for reducing sugars. Different brands of yeast cake and dry yeast can be used. Excellent recoveries have been obtained when varying quantities of nonfat dry milk solids were added to water bread. Likewise, results determined by two different laboratories on samples of bread of varying concentrations of lactose or nonfat dry milk solids showed good agreement between the two laboratories and also with the theoretical values.

Methods for the estimation of nonfat dry milk solids in bread are generally based upon the determination of lactose by selective fermentation with baker's yeast. Among the procedures utilizing this principle are those of Snethlage (7), Meyer (5), Schut and DeJong (6), and Magraw and Copeland (4). The last procedure was developed in this laboratory and has been adopted in *Cereal Laboratory Methods* (2). While it gives satisfactory results, it is time consuming and somewhat objectionable because it requires the use of hydrogen sulfide.

A shorter and less tedious method is presented in this report. Extraction of the lactose is accomplished with dilute ethanol, a procedure which eliminates the starches and nearly all the proteins. Consequently, it is not necessary to use alpha-amylase or repeated protein precipitations in subsequent operations. Also, by using a small aliquot along with continuous agitation, the time required for complete fermentation of non-lactose reducing sugars is reduced to two and one-half hours. Finally, substitution of the micro-method of Somogyi for the Munson-Walker method for lactose determination further decreases the time required per analysis.

Materials and Methods

Reagents. 1. Yeast suspension. This was prepared according to the procedure of Jones (3). Commercial baker's yeast was washed by

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Contribution from American Dry Milk Institute, Inc., Chicago, 1, Illinois.

centrifuging with three to four times its volume of distilled water until the supernatant liquid was clear. The yeast was finally diluted to a 25% suspension and stored in a refrigerator at 0° to 4°C. for 24 hours before use. Preparations as old as one week could still be used.

2. Yeast nutrient solution. 1.7 g. bacto-peptone (Difco Laboratories), 0.50 g. dipotassium phosphate, and 0.33 g. magnesium sulfate heptahydrate were dissolved in water and diluted to 100 ml.

3. Protein precipitant. 50 g. sodium tungstate and 6 g. disodium phosphate were dissolved in 200 ml. distilled water. After adding slowly 220 ml. of 2 *N* hydrochloric acid, the solution was mixed and diluted to 500 ml.

4. Somogyi's reagent (8). 12 g. Rochelle salt, 20 g. anhydrous sodium carbonate, and 25 g. sodium bicarbonate were dissolved in about 500 ml. distilled water. To this was added with stirring 6.5 g. copper sulfate pentahydrate previously dissolved in 100 ml. water. In a separate container, 10 g. potassium iodide, 0.800 g. potassium iodate, and 18 g. potassium oxalate were dissolved in about 200 ml. water. The two solutions were mixed and diluted to 1 l.

5. Sodium thiosulfate, 0.005 *N*. Since this solution cannot be kept unchanged for long, it was prepared every day by diluting freshly standardized 0.1 *N* solution.

6. Sodium hydroxide, 0.5 *N*.

7. Sulfuric acid, 2 *N*.

8. Starch solution, 1%.

Procedure. The bread to be analyzed was sliced, air-dried, and then crushed to fine particles. The moisture content of the air-dried sample was determined by the A.O.A.C. vacuum oven method (1). A sample of 15 g. of the air-dried bread was weighed into a 200 ml. volumetric flask containing 60 ml. water. After thorough mixing, 35 ml. 95% ethanol were added and the sample immersed in a boiling water bath for 15 minutes. When cooled to room temperature, the content was diluted to volume with 95% ethanol, transferred to a centrifuge bottle, and centrifuged for 10 minutes at approximately 1,000 r.p.m. One hundred and fifty milliliters of the supernatant liquid were evaporated to about 40 ml. and then diluted to 100 ml. in a volumetric flask.

For fermentation, 10 ml. of this bread extract were transferred to a 50 ml. Erlenmeyer flask; 6 ml. yeast suspension and 5 ml. yeast nutrient solution were added. A blank test was made using 10 ml. of water in place of the bread extract. The flask was stoppered with a one-hole rubber stopper fitted with a piece of 6 mm. glass tubing about 10 cm. long and shaken at a moderate rate for 2.5 hr. in a constant temperature water bath at 30°C. The fermented sample was then

transferred to a 50 ml. centrifuge tube and centrifuged for 10 minutes at approximately 1,000 r.p.m. The supernatant layer was decanted into a 50 ml. volumetric flask and the residue washed twice with 10 ml. portions of water. The washings were combined with the decanted liquid. To this solution was next added, with shaking, 2.5 ml. protein precipitant. After diluting to volume and mixing, the mixture was filtered, discarding the first few milliliters of the filtrate.

For lactose determination, 5.00 ml. of the clear filtrate were pipetted into a Pyrex culture tube (22 × 175 mm) and neutralized to the phenol red end-point with 0.5 *N* sodium hydroxide. To this, 5.00 ml. Somogyi's reagent and two drops of benzene were added. The tube was capped with a glass bulb and immersed in a vigorously boiling water bath for exactly 15 minutes after which the contents were cooled without agitation in a cold water bath. Then, 2.5 ml. 2 *N* sulfuric acid were added. When the cuprous oxide precipitate was completely dissolved, the excess iodine was titrated with 0.005 *N* sodium thiosulfate using starch as an indicator. The difference between the titration value of the blank and that of the sample was referred to a standard curve to determine the quantity of lactose present. The reference curve was established by testing 5.00 ml. portions of lactose solutions containing from 0 to 5 mg. of lactose hydrate with 5.00 ml. portions of Somogyi's reagent and plotting the difference between the titration value of the blank and that of the lactose solution against the corresponding lactose content.

From the amount of lactose found in the 5.00 ml. aliquot of the fermented sample, the percentage of lactose present in the moisture-free bread can be calculated as follows:

$$\% \text{ lactose} = \frac{187}{150} \times \frac{100}{10} \times \frac{50}{5} \times \frac{100 \times L}{15(100 - M)} \times 100 = \frac{8.33 \times L \times 10^4}{100 - M}.$$

"L" is the grams of lactose in the 5.00 ml. aliquot and "M" is the per cent moisture in the air-dried bread. The factor 187 is the total volume of dilute alcohol in 200 ml. of bread suspension, using 13 ml. as the volume occupied by 15 g. of air-dried bread, a factor employed by Magraw and Copeland (4). A blank of 0.35% lactose was subtracted from the final result. This is a correction for the amount of non-fermentable reducing material present in bread containing no milk and is about the same as that found by the above workers (4). To convert to nonfat dry milk solids, which is the usual source of lactose in bread, the per cent of lactose is multiplied by two, since the average lactose content of nonfat dry milk solids is approximately 50%. A sample of dry bread of known lactose content may be run simultaneously with unknown breads, if desired, to check the reliability of each series of analyses.

Results and Discussion

Except where otherwise noted, samples of bread for this study were prepared by our baking laboratory, using the following formula. The straight-dough technique was followed:

Flour	300 g.	Yeast food	0.75 g.
Water	210 g.	Malt	1.5 g.
Sugar	12 g.	Shortening	6 g.
Salt	6 g.	Nonfat dry milk solids, variable	
Yeast	6 g.		

In accordance with general baking practice, the percentage of nonfat dry milk solids or lactose added is expressed as per cent of flour used.

Using an extract prepared from a bread containing 6% nonfat dry milk solids, the time necessary for complete fermentation of non-lactose reducing sugars was determined by fermenting 10 ml. portions for varying periods at 30°C. with shaking. From the data plotted in

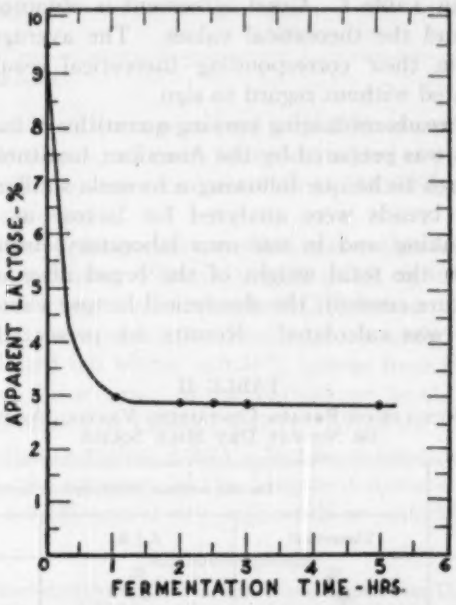

Fig. 1. Fermentation time necessary for the complete destruction of non-lactose reducing substances on extract of bread containing 6% of nonfat dry milk solids (2.89% lactose).

Fig. 1 it can be noted that fermentation is practically complete after 1 hour. A 2½ hour period was chosen to insure complete fermentation. In the absence of agitation, a much slower rate of fermentation resulted.

Recovery experiments were made by adding to an air-dried water-bread, nonfat dry milk solids (51.2% lactose) in amounts to give 0,

TABLE I

RECOVERY OF NONFAT DRY MILK SOLIDS[1] ADDED TO WATER BREAD

Nonfat dry milk solids added	Lactose, theoretical	Lactose found		
		Trial 1	Trial 2	Trial 3
%	%	%	%	%
0	0	0	0.11	0.17
2	1.02	1.07	1.12	1.11
4	2.05	2.13	2.20	2.20
6	3.08	3.21	3.21	3.19
8	4.10	4.09	4.09	3.91
12	6.15	5.92	6.13	6.13

[1] Containing 51.2% lactose by analysis.

2, 4, 6, 8, and 12% nonfat milk solids. These mixtures were analyzed for lactose by the present method. Results of three separate trials are presented in Table I. Good agreement is obtained between the experimental and the theoretical values. The average deviation of all results from their corresponding theoretical results is 0.092% lactose, calculated without regard to sign.

A series of breads containing varying quantities of lactose or nonfat dry milk solids was prepared by the American Institute of Baking by the sponge dough technique following a formula similar to that given above. These breads were analyzed for lactose at the American Institute of Baking and in our own laboratory, using the present method. From the total weight of the bread after air drying and from the moisture content, the theoretical lactose value on the moisture-free basis was calculated. Results are presented in Table II.

TABLE II

COLLABORATIVE RESULTS ON BREADS CONTAINING VARYING AMOUNTS OF LACTOSE OR NONFAT DRY MILK SOLIDS

Lactose content[1]	Lactose content (moisture-free basis)		
	Theoretical	A.I.B.	ADMI
%	%	%	%
0	0	—	0.0
0	0	0.12	0.01
2	1.96	—	1.75
4	3.82	3.67	3.60
6	5.96	5.84	5.48, 5.42
1	0.97	0.80	0.86
2	1.91	1.56	1.74
3	2.89	2.71	2.84, 2.86
4	3.98	3.75	3.80

[1] In the first five samples listed lactose was added; in the last four nonfat dry milk solids was used.

The agreement between results from the two laboratories, as well as between the observed and the theoretical results, is satisfactory.

In most of these experiments Fleischmann baker's yeast (one-pound size) was used. However, when other brands of yeast, both in the cake form and in the dry form, were tried, they were found to be equally satisfactory as indicated by the data in Table III for two

TABLE III

RESULTS WITH DIFFERENT BRANDS OF YEAST

Yeast	Lactose found, % (moisture-free basis) 4% Lactose bread	8% ndms[1] bread
Fleischmann (pound cake)	4.14	3.98
Fleischmann (⅗ oz. cake)	3.72	4.15
Fleischmann (1 oz. cake)	4.00	4.20
Fleischmann (dry)	4.16	4.15
Red Star (⅔ oz. cake)	4.11	4.13
Red Star (dry)	3.95	3.95
National (2 oz. cake)	4.16	3.89
National (dry)	4.16	3.97

[1] Nonfat dry milk solids.

breads analyzed on different occasions with different samples of yeasts. In using the dry yeasts, sufficient water was added to give a suspension of approximately the same concentration as the 25% suspension prepared from yeast cakes.

The precision of the present method was estimated by analyzing a white bread containing 6% nonfat milk solids eight separate times. The standard deviation was found to be 0.12% lactose, which, assuming normal distribution, would mean that approximately 95% of all determinations should fall within ±0.24% lactose from the mean value. An indication of the accuracy of the method can be obtained from the recovery results shown in Tables I and II. With the exception of two results, all results are within ±0.24% lactose of the theoretical values. In comparison, the accuracy of the Magraw-Copeland procedure has been given as ±0.5% nonfat dry milk solids or ±0.25% lactose.

Acknowledgment

Grateful acknowledgment is given to the following persons: To Mr. S. Crespo of the American Institute of Baking, Chicago, Illinois for baking and analyzing the nine samples of bread used in the collaborative study; to Mr. C. DeBrower of our laboratory for baking the other breads used; and to Mr. V. E. Marx, in charge of the Bakery Division of this Institute, for his helpful suggestions and comments.

Literature Cited

1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and tentative methods of analysis, 6th ed. Washington, D. C. (1945).
2. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Cereal laboratory methods. 5th ed. Am. Assoc. Cereal Chemists: St. Paul, Minn. (1947).

3. JONES, T.S.G. The determination of lactose and glucose in milk. J. Dairy Res. 7: 41–46 (1936).
4. MAGRAW, D. A., and COPELAND, L. E. Determination of milk solids not fat in bread. Cereal Chem. 13: 541–549 (1936).
5. MEYER, W. Milk sugar in bread. Chem. Weekblad 30: 317–319 (1933).
6. SCHUT, W., and DEN DOOREN DE JONG, L. E. Lactose bepaling in brood. Chem. Weekblad 22: 518–520 (1925).
7. SNETHLAGE, M. W. F. Lactose bepaling in brood. Chem. Weekblad 23: 578–580 (1926).
8. SOMOGYI, M. Notes on sugar determination. J. Biol. Chem. 70: 599–612 (1926).

INHIBITION OF BETA-AMYLASE BY CYANIDE[1]

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ABSTRACT

Addition of sodium cyanide to malt extract solutions markedly inhibited the saccharification produced by these extracts. The cyanide addition had no effect on alpha-amylase activities but lowered apparent beta-amylase activities of these extracts. Sodium cyanide completely inhibited the saccharifying activities of extracts from ungerminated soybeans, wheat and barley, and of solutions of a commercial beta-amylase. Cyanide, therefore, has a differential effect upon amylase complexes, inhibiting the beta-amylase. This inhibition is complete when beta-amylase is the only amylolytic enzyme present, but apparently not with extracts of malt.

Inhibition or destruction of enzyme activity by chemical reagents is frequently encountered. An inhibiting agent may sometimes be employed to advantage in the study of enzyme systems for the inhibition of the activity of certain enzyme components without affecting others.

In the course of studies on the enzyme systems of bacteria and molds, interest arose in finding substances which would have a differential inhibiting effect upon components of the amylase complex. Although a number of reports on amylase inhibitors have appeared, in most cases these have been unidentified substances of natural origin (1, 5, 7, 14). However, Purr (12) found that ascorbic acid inhibited beta-amylases but did not affect the alpha-amylases of plants. Hanes (2), Weidenhagen and Lu (15), and Janicki (3) likewise found that ascorbic acid inhibited the action of beta-amylase, and Hanes reported that reductone and dihydroxymaleic acid also inhibited beta-amylase.

Cyanides have frequently been employed for inhibiting various enzymes, particularly in metabolic studies. There are few reports on

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the effect of cyanide upon amylases, especially upon the effect of cyanide upon the individual components of amylase complexes. Myrbäck (8) found that potassium cyanide in small amounts strongly inhibited the activity of salivary amylase, whereas equimolar concentrations of hydrogen cyanide caused much less inhibition. However, 0.1 *N* hydrogen cyanide almost completely destroyed the activity of the enzyme. Ninomiya (10) found that salivary amylase was somewhat inhibited by potassium cyanide or potassium thiocyanate, even in concentrations as low as 0.0005 *M*. Nguyen-Van-Thoai and Silhol-Bernere (9) reported that purified pancreatic amylase treated at pH 6.8 at 40°C. for one-half hour with 0.001 to 0.1 *M* potassium cyanide was not affected, whereas the crude enzyme was inhibited by this reagent at very low concentrations.

Since salivary amylase and pancreatic amylases are predominately of the alpha-amylase type, tests were made on the inhibiting action of cyanide upon certain enzyme extracts having alpha-amylase activity. Preliminary tests showed that cyanide markedly inhibited the amylolytic activity of malt extracts but had relatively little effect upon fungal amylase solutions. Further work, reported in this paper, showed that cyanide inhibits beta-amylase without affecting activities of other components of the amylase complex.

Materials and Methods

The amylolytic materials employed in this investigation included samples of malts, grains, and a commercial beta-amylase preparation. Three malts, kindly supplied by Dr. Eric Kneen of the Kurth Malting Company, of widely varying alpha-amylase and beta-amylase activities were used. One of these, malt A, was a "high-kilned malt," low in beta-amylase and limit dextrinase activities. Two varieties of soybeans and one sample each of wheat and barley were supplied by the Agronomy Department of Iowa State College. The commercial beta-amylase preparation was "beta-amylase for analytical purposes" purchased from Wallerstein Laboratories.

Extracts were prepared from the malts and grains for testing in the following manner: The materials were finely ground in a burr mill and 5 g. of each extracted with 100 ml. of water for 1 hour at 30° C., with frequent shaking. The extracts were then centrifuged and filtered. Where cyanide treatment was employed, 50 mg. of sodium cyanide was added to 50 ml. of extract, which corresponds to 0.1% or 0.02 *M* concentration of the cyanide, and the solution held for 1 hour at 30° C.

Alpha-amylase determinations were made by the method of Sandstedt, Kneen, and Blish (13) using the cobalt-chromate color

standard of Olson, Evans, and Dickson (11). Results of the analyses were calculated in terms of the alpha-dextrinogenic units of the former authors, representing the number of grams of soluble starch which, under the influence of an excess of beta-amylase, are dextrinized by one gram of malt in one hour at 30°C.

Overall saccharification values and beta-amylase activities were determined by the method of Kneen and Sandstedt (4). The results were calculated in terms of saccharogenic units, representing the number of grams of soluble starch converted to maltose by one gram of material in one hour at 30°C., and beta-amylase units, representing the number of grams of soluble starch converted to maltose by the beta-amylase of one gram of material in one hour at 30°C. Independent tests showed that the amounts of cyanide employed affected the quantities of apparent maltose found by the ferricyanide method by from 0 to 2%, depending upon the sugar concentration. Such differences are not significant for the comparisons involved in this investigation.

Results and Discussion

The procedures devised by Sandstedt, Kneen, and Blish (13) and by Kneen and Sandstedt (4) for differential estimation of alpha-amylase and beta-amylase in malt were adapted to the investigation of the inhibiting action of cyanide upon these components of malt amylase. Extracts of the three malts were prepared and portions of each were treated with sodium cyanide as described above. The cyanide-treated and untreated extracts were then analyzed. The data for the malt samples are given in Table I.

TABLE I

Effect Upon Amylolytic Activity of Treating Aqueous Extracts of Malts with 0.1% Sodium Cyanide

Malt sample	Untreated extract			Cyanide-treated extract		
	Alpha-dextrinogenic units	Saccharogenic units	Beta-amylase units	Alpha-dextrinogenic units	Saccharogenic units	Beta-amylase units
A	34	5.34	3.65	34	2.66	0.97
B	64	19.37	16.33	63	14.32	11.33
C	46	9.67	7.42	45	7.36	5.16

The results of this experiment indicated that cyanide had little if any effect upon the alpha-amylase activity of the malt extracts, but markedly lowered the overall saccharification produced by the extracts. However, apparent residual beta-amylase activity, as calculated from the analytical data, still existed.

The effect of cyanide treatment of aqueous extracts of soybeans, wheat, and barley was then investigated. Since extracts of these ungerminated grains are good sources of beta-amylase, while alpha-amylase is essentially absent, only saccharogenic values were determined on the cyanide-treated and untreated extracts. Simultaneously with the grain extracts, solutions of three different concentrations of the commercial beta-amylase were prepared, a portion of each treated with 0.1% sodium cyanide in the same manner as for the grain extracts, and saccharogenic values determined on the solutions. Data from these determinations are given in Table II.

TABLE II

EFFECT UPON AMYLOLYTIC ACTIVITY OF TREATING SOLUTIONS FROM SOURCES OF BETA-AMYLASE WITH 0.1% SODIUM CYANIDE

Sample	Saccharogenic units	
	Untreated	Cyanide-treated
Soybean A	10.03	0
Soybean B	9.00	0
Wheat	4.90	0
Barley	5.56	0
Commercial beta-amylase:		
0.2% solution	2.66[1]	0
0.5% solution	6.67[1]	0
1.0% solution	13.55[1]	0

[1] Units per ml. of solution.

The results presented in Table II show conclusively that beta-amylase from the sources tested is completely inhibited or destroyed by sodium cyanide. Even solutions of the commercial beta-amylase in the relatively high concentration of 1.0% showed no saccharifying activity after cyanide treatment.

Referring again to the data of Table I, it may be noted that although overall saccharification was markedly lowered by treatment of the malt extracts with cyanide, apparent residual beta-amylase activity was still appreciable. This was especially true for malts B and C. Since cyanide completely destroyed the beta-amylase of the ungerminated grains and commercial beta-amylase, it would be assumed that the beta-amylase activity of the malts should also be completely inhibited. It is possible, however, that other constituents in the extracts may exert a protective action on the beta-amylase of malts. Probably other components of the malt amylase system, such as limit dextrinase, are not inactivated and together with the alpha-amylase are responsible for much of the saccharification produced. The apparent residual beta-amylase activity may possibly be due, at least in part, to limit dextrinase. The contribution which limit dex-

trinase makes to total saccharification needs further investigation to elucidate this point. However, the hypothesis is strengthened by the fact that the apparent beta-amylase activity for malt A after cyanide treatment was relatively low. Malt A was a "high-kilned malt," so treated for destruction of beta-amylase and limit dextrinase. Such treatment does not entirely eliminate these components, but markedly reduces them according to Kneen and Spoerl (6). The data show relatively low beta-amylase activity for this malt before cyanide treatment, and very low residual apparent beta-amylase activity after cyanide treatment.

It may be concluded from the work reported in this paper that cyanide has a differential effect upon amylolytic enzyme complexes, inhibiting only the activity of beta-amylase. When beta-amylase is present as the sole amylolytic component it is completely inhibited by cyanide. The apparent beta-amylase activity of malts is apparently only partially inhibited by cyanide. Further work will be necessary to fully establish the degree of inhibition of beta-amylase in malts and in other mixtures of the several amylase components.

Literature Cited

1. Bowman, D. E. Amylase inhibitor of navy beans. Science **102:** 358–359 (1945).
2. Hanes, C. S. The reversible inhibition of β-malt-amylase by ascorbic acid and related compounds. Biochem. J. **29:** 2588–2603 (1935).
3. Janicki, J. Oxydo-Reduktionsprozesse bei der Hemmung bezw. Aktivierung der Amylase. Die Rolle der Ascorbinsäure. Enzymologia **7:** 182–192 (1939).
4. Kneen, E., and Sandstedt, R. M. Beta-amylase activity and its determination in germinated and ungerminated cereals. Cereal Chem. **18:** 237–252 (1941).
5. Kneen, E., and Sandstedt, R. M. Distribution and general properties of an amylase inhibitor in cereals. Arch. Biochem. **9:** 235–249 (1946).
6. Kneen, E., and Spoerl, Joan. The limit-dextrinase activity of barley malts. Am. Soc. Brewing Chemists, Proc. **1948:** 20–27.
7. Militzer, W., Ikeda, Carol, and Kneen, E. The preparation and properties of an amylase inhibitor of wheat. Arch. Biochem. **9:** 309–320 (1946).
8. Myrbäck, K. Über Verbindungen einiger Enzyme mit inaktivierenden Stoffen. II. Z. physiol. Chem. **159:** 1–84 (1926).
9. Nguyen-Van-Thoai, and Silhol-Bernère, Madeleine. Sur les caractères de l'amylase pancréatique purifiée. Compt. rend. **223:** 961–962 (1946).
10. Ninomiya, H. Beitrag zur Kenntnis der Speichelamylase. II. Mitteilung: Über die Spaltung der Speichelamylase durch Proteasen und über ihren Kohlenhydrat- und Eisengehalt. J. Biochem. (Tokyo) **31:** 421–428 (1940).
11. Olson, W. J., Evans, Ruth, and Dickson, A. D. A modification of the Kneen and Sandstedt methods for the determination of alpha- and beta-amylases in barley malt. Cereal Chem. **21:** 533–539 (1944).
12. Purr, A. The influence of vitamin C (ascorbic acid) on plant and animal amylases. Biochem. J. **28:** 1141–1148 (1934).
13. Sandstedt, R. M., Kneen, E., and Blish, M. J. A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. **16:** 712–723 (1939).
14. Sukhorukov, I., Kling, E., and Ovcharov, K. E. The effect of *Phytophthora infestans* de Bary on the enzymes of affected plants. Compt. rend. acad. sci. U.R.S.S. **18:** 597–602 (1938).
15. Weidenhagen, R., and Lu, Pao-chung. Relation between vitamin C and enzymic sucrose cleavage. Z. Wirtschaftsgruppe Zuckerind. **86:** Tech. T1. 240–253 (March 1936). Original not seen, C.A. **30:** 6013 (1936).

EXPERIMENTS WITH AMMONIUM PERSULFATE AS A FLOUR MATURING AGENT [1]

J. M. DOTY AND R. C. SHERWOOD [2]

ABSTRACT

Ammonium persulfate in bread flours at levels of 1 to 20 g. per 100 pounds of flour showed a definite maturing action. Loaf volumes and crumb texture scores were improved by ammonium persulfate in spring, winter, and west coast bread flours. Ammonium persulfate treated flours showed good storage properties and compared favorably in baking properties with nitrogen trichloride treated flours, both freshly milled and after several months' storage.

Ammonium persulfate additions to flour did not affect the thiamine and riboflavin content of enriched flour. No deleterious effect could be observed on the vitamin content of persulfate-treated, enriched flour after storage, either in the flour or in the bread made from the flour.

Ammonium persulfate has been used as a flour maturing agent in the production of bread for many years in Europe. Very few reports appear in the literature. Kent-Jones and Amos (1) have reported its maturing properties, and its use in England and on the Continent for many years, stating that persulfates cause improvement in elasticity and stability of dough, and their use results in superior breads. Because certain changes are being made in the use of maturing gases in the production of flour, investigations into the use of ammonium persulfate in maturing flour are herewith reported.

Ammonium persulfate is a powerful oxidizing agent, containing 7% active oxygen. It is a white crystalline compound which can be blended with relatively inert compounds and added directly to flour or flour doughs. Since the persulfate is water soluble, it goes into solution promptly when a dough is mixed and exerts its oxidizing action without delay.

This report shows the use of ammonium persulfate in maturing bakers flour made from hard red winter wheat, spring wheat, and west coast hard wheats.

Materials and Methods

Baking results are reported on a modified A.A.C.C. procedure using 100 g. of flour per loaf. The ingredients used in the modified formula include yeast, salt, sugar, shortening, milk, malt, and flour. All results

[1] Manuscript received May 16, 1949.
[2] Sterwin Chemicals Inc. Work done in Doty Laboratories, Kansas City, Missouri.

obtained on 100 g. flour loaves were verified by the sponge-dough procedure producing regular 1-pound commercial-type loaves.

Over 50 southwestern winter wheat bakers flours have been treated with ammonium persulfate and compared with flours given regular commercial nitrogen trichloride maturing. Many spring wheat bakers flours and west coast bakers flours have also been compared in this manner. For this report, a typical example has been chosen from each of the three groups of bakers flours tested.

Ammonium persulfate was added to flour both in the dry form and in solution at the dough mixing stage. The dry form was a fine-ground mixture of 50% ammonium persulfate and 50% inert carrier such as calcium sulfate.

All flour used in storage tests was stored in cloth bags on open shelves at room temperature in the laboratory. Sacks were opened for analytical work, re-tied, and returned to shelf for further storage.

Results and Discussion

Southwest bakers flour untreated and with various increments of nitrogen trichloride or ammonium persulfate are reported in Table I. The optimum nitrogen trichloride treatment for this flour is in the neighborhood of 1 g. per 100 pounds of flour, which compares closely with 4 g. of ammonium persulfate per 100 pounds of flour. Loaf volumes, texture scores, and over-all bread scores show similar results

TABLE I

BAKING RESULTS ON HARD WINTER WHEAT BAKERS FLOUR COMPARING AMMONIUM PERSULFATE AND NITROGEN TRICHLORIDE AS MATURING AGENTS

Treatment per 100 lbs. flour	Loaf volume	Texture score	Total bread score
	cc.		
Unmatured	635	50	59
½ gram N.T.[1]	735	70	78
1 gram N.T.	840	90	95
2 grams N.T.	800	70	73
2 grams A.P.[2]	750	80	86
4 grams A.P.	850	89	95
8 grams A.P.	810	75	78

[1] N.T.—Nitrogen Trichloride.
[2] A.P.—Ammonium Persulfate.

between the maturing action of nitrogen trichloride and ammonium persulfate. These data are typical of results obtained on over 50 samples of southwestern winter wheat flours.

The effect of various increments of nitrogen trichloride and ammonium persulfate on untreated spring wheat bakers flour is reported in Table II. The optimum nitrogen trichloride treatment is about 1.0 g.

TABLE II

BAKING RESULTS ON SPRING WHEAT BAKERS FLOUR COMPARING AMMONIUM PERSULFATE AND NITROGEN TRICHLORIDE AS MATURING AGENTS

Treatment per 100 lbs. flour	Loaf volume	Texture score	Total bread score
	cc.		
Unmatured	700	65	71
½ gram N.T.[1]	875	90	91
1 gram N.T.	880	97	97
1½ grams N.T.	800	73	77
2 grams A.P.[2]	850	81	85
3 grams A.P.	870	92	94
4 grams A.P.	860	91	92

[1] N.T.—Nitrogen Trichloride.
[2] A.P.—Ammonium Persulfate.

per 100 pounds of flour and the optimum treatment with ammonium persulfate, somewhere near 3 g. per 100 pounds of flour. Here again loaf volumes, texture scores, and over-all bread scores are similar for the optimum treatments with nitrogen trichloride or ammonium persulfate. These data are typical of many spring wheat flours tested in like manner.

Pacific coast bakers flour with various increments of nitrogen trichloride or ammonium persulfate is reported in Table III. Results are similar to those obtained on southwest bakers flour.

From these baking results, it is apparent that ammonium persulfate has definite value in artificially aging bread flours. In order to determine whether ammonium persulfate has practical application, storage tests were conducted. Finely-ground ammonium persulfate was mixed with an equal quantity of a mixture of calcium sulfate and calcium phosphate in order to make a free-flowing material suitable for mixing in flour. The flour used for the storage tests required 6 g. of ammonium

TABLE III

BAKING RESULTS ON WEST COAST BAKERS FLOUR COMPARING AMMONIUM PERSULFATE AND NITROGEN TRICHLORIDE AS MATURING AGENTS

Treatment per 100 lbs. flour	Loaf volume	Texture score	Total bread score
	cc.		
Unmatured	610	50	57
½ gram N.T.[1]	715	69	75
1 gram N.T.	755	88	89
2 grams N.T.	705	75	80
2 grams A. P.[2]	700	70	75
4 grams A.P.	760	89	88
8 grams A.P.	710	77	80

[1] N.T.—Nitrogen Trichloride.
[2] A.P.—Ammonium Persulfate.

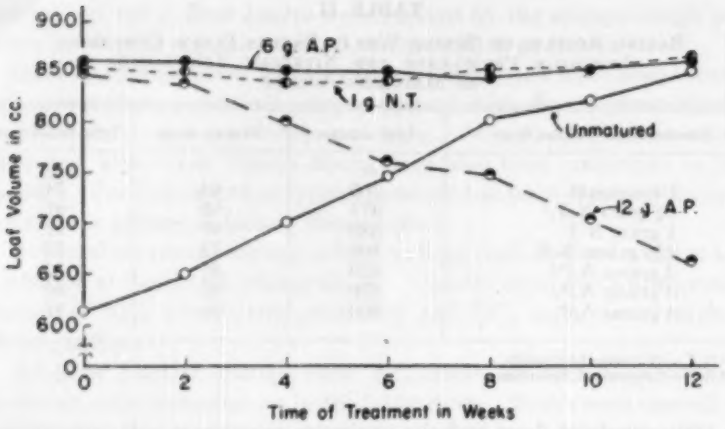

FIG. 1. Loaf volumes of flours during storage.

persulfate per 100 pounds of flour to produce optimum baking results. Using the 50% mixture, the flour was treated at two different levels, one representing 6 g., and the other 12 g. of ammonium persulfate per 100 pounds of flour. Twice optimum treatment was used deliberately in the second flour to determine the degree of damage resulting from over-maturing with ammonium persulfate when fresh and after storage.

The baking results obtained with these two flours compared with the same flour untreated, and treated with nitrogen trichloride, are reported in Fig. 1. The 6 g. ammonium persulfate treated flour and

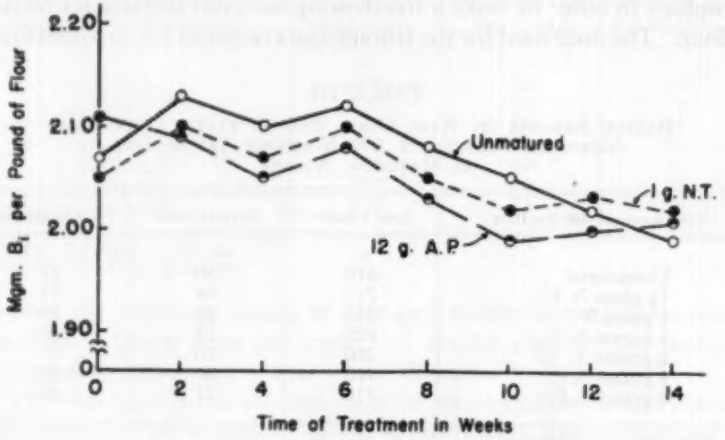

FIG. 2. Thiamine content of flours during storage.

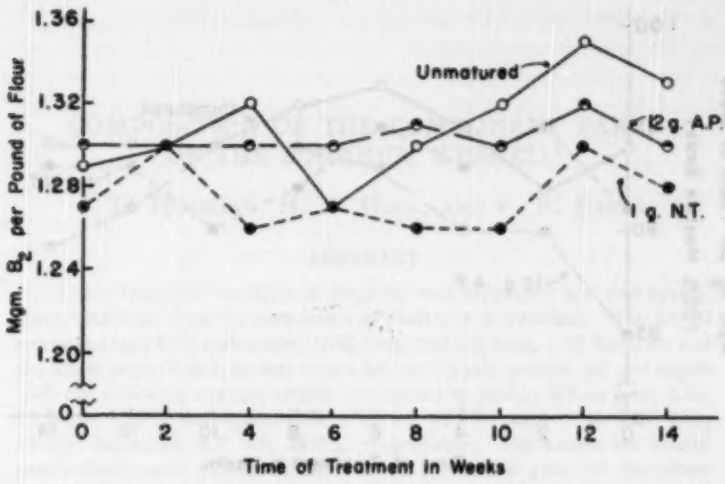

FIG. 3. Riboflavin content of flours during storage.

the nitrogen trichloride treated flour paralleled very closely over three months' storage period. As would be expected, the over-treatment with persulfate showed a decrease in loaf volume and the untreated flour showed an increase in loaf volume over the three months' period.

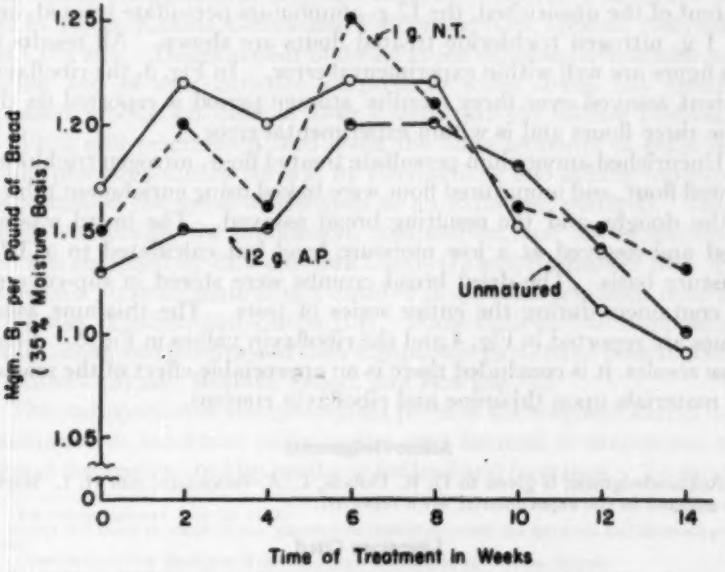

FIG. 4. Thiamine content of bread made with stored flour.

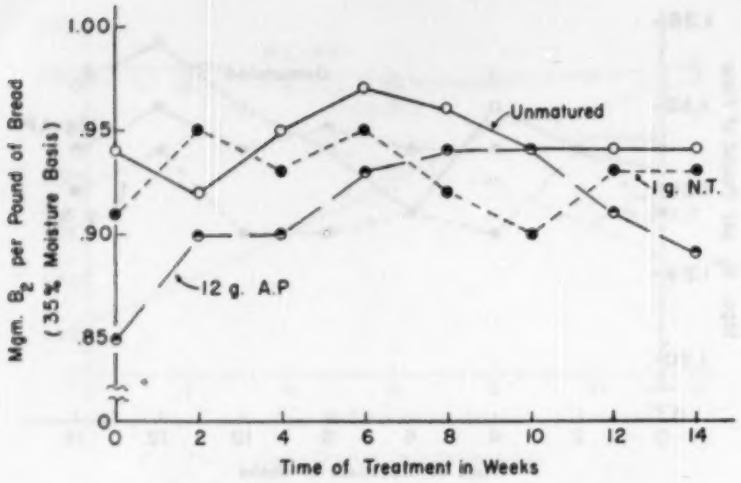

FIG. 5. Riboflavin content of bread made with stored flour.

The optimum ammonium persulfate treated flour produced satisfactory baking results over several months' storage period.

To determine the effect of ammonium persulfate on the vitamins of enriched flour, the flours of Fig. 1 were also assayed over the storage period for thiamine and riboflavin content. In Fig. 2, the thiamine content of the unenriched, the 12 g. ammonium persulfate treated, and the 1 g. nitrogen trichloride treated flours are shown. All results in this figure are well within experimental error. In Fig. 3, the riboflavin content assayed over three months' storage period is reported on the same three flours and is within experimental error.

Unenriched ammonium persulfate treated flour, nitrogen trichloride treated flour, and unmatured flour were baked using enrichment tablets in the doughs and the resulting bread assayed. The bread was air dried and assayed at a low moisture level but calculated to a 35% moisture basis. The dried bread crumbs were stored in slip-covered tin containers during the entire series of tests. The thiamine assay values are reported in Fig. 4 and the riboflavin values in Fig. 5. From these results, it is concluded there is no appreciable effect of the maturing materials upon thiamine and riboflavin content.

Acknowledgments

Acknowledgment is given to D. K. Dubois, C. A. Brockman, and H. L. Marks, who assisted in the experimental work reported.

Literature Cited

1. KENT-JONES and AMOS. "Modern Cereal Chemistry," 4th Edition, 1947, Page 250.

COMPOSITION OF THE COMPONENT PARTS OF THE SORGHUM KERNEL[1,2]

J. E. Hubbard, H. H. Hall, and F. R. Earle

ABSTRACT

Grain from five varieties of sorghum was separated into endosperm, germ, and bran, and the proportion of each was determined. The kernel contains about 82% endosperm, 10% germ, and 8% bran. All fractions and the whole grain of each variety were analyzed for ash, protein, oil, and starch with the following average results (moisture-free basis): Whole grain 1.65, 12.3, 3.6, 73.8%; endosperm 0.37, 12.3, 0.6, 82.5%; germ 10.36, 18.9, 28.1, 13.4%; bran 2.02, 6.7, 4.9, 34.6%, respectively. The assays for niacin, panthothenic acid, riboflavin, biotin, and pyridoxine gave for the whole grain 45.3, 10.4, 1.3, 0.20, 4.7 μg./g.; endosperm 43.7, 8.7, 0.9, 0.11, 4.0 μg./g.; germ 80.7, 32.2, 3.9, 0.57, 7.2 μg./g.; and bran 44.0, 10.0, 4.0, 0.35, 4.4 μg./g., respectively. The whole grain was analyzed for crude wax by a new method and was found to contain 0.32%.

Grain sorghums have been grown extensively for many years in areas of the Southwest where climatic conditions make corn production uncertain. Such adaptation of sorghum makes it important as forage and feed in Texas, Kansas, Oklahoma, California, New Mexico, Nebraska, and Colorado. Development of dwarf types suitable for machine harvesting and of new varieties adapted to wider areas has steadily increased production until in 1949 it exceeded 152 million bushels.

While still primarily a feed crop, the large amounts of grain sorghums processed during the war by the distilling and milling industries demonstrated the value of the crop as an industrial material. Information concerning the production, composition, and use of sorghums has been compiled by Edwards and Curtis (7) and Hightower (8). The B-complex vitamin content of a number of varieties has been reported by Tanner, Pfeiffer, and Curtis (14). Some data on the composition of kafir, milo, and feterita and their component parts have been reported by Bidwell (3) and Bidwell, Bopst, and Bowling (4).

The composition of the component parts of the sorghum kernel is of importance to industries utilizing the crop because it determines the value of derivatives and by-products for feed and food uses. To supply

[1] Manuscript received May 10, 1950.
Report of a study in which certain phases were carried on under the Research and Marketing Act of 1946.
[2] Contribution from Northern Regional Research Laboratory, Peoria, Illinois.
One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

further information on this, separation and analyses were made on grain of several varieties of sorghums.

Determinations were made of the amount of endosperm, germ, and bran, and these fractions were analyzed for ash, protein, oil, and starch, and five of the B-complex vitamins. Varieties selected for analysis are in commercial production and include Cody (waxy kafir), Pink Kafir, and the three yellow milos—Westland, Midland, and Martin. Four of the varieties selected are representative of the sorghum producing area of Kansas—Westland being a composite of samples from 11 counties, Midland a composite from 12 counties, Cody a composite from 11 counties, and Pink Kafir from 3 counties. Martin was from a large lot of Texas certified seed grown near San Antonio. All samples were grown in 1945.

Methods

As a check on the uniformity of the samples and the accuracy of the separations, each sample was divided into four portions of approximately 20 g. each. Each portion was inspected and picked to include only sound whole kernels. Distilled water at room temperature was used to soak the grain sufficiently to facilitate removal of the bran and separation of the germ from the endosperm. Three to seven minutes were usually sufficient when the initial moisture content of the grain was 12 to 14%. Fractions were separated by hand with a scalpel, checked visually for completeness of separation, and air dried before weighing. The replications for physical proportions agreed within each variety within about 1% and were combined for chemical analysis. It was estimated initially that 5 g. of bran, the smallest fraction, would provide sufficient material for the determination of oil, protein, and vitamins, and, accordingly, only enough grain was separated to provide that quantity.

Whole grain and endosperm fractions were ground for analysis in a special laboratory hammer mill (10) with a 1/16-inch perforated screen. Bran fractions were ground in a micro Wiley mill with a 20-mesh screen. Germ was analyzed without preliminary grinding.

The grain and the separated fractions were analyzed for moisture, ash, nitrogen, oil, and starch. Methods of the Association of Official Agricultural Chemists (1) were used to determine moisture, ash, and nitrogen. Oil was determined by the A.O.A.C. method for oil in cottonseed, except that the samples of germ were extracted about six hours, ground in a mortar, and then re-extracted overnight. Starch was determined by the Clendenning polarimetric method (5) with uranyl acetate being used in place of stannic chloride as the protein precipitant.

Crude wax was determined by a simple procedure by use of hot benzene as solvent. Thirty to 60 g. samples of whole grain were placed

on small squares of cheesecloth and the corners of the cloth gathered to make a bag. The sample was dipped for ten seconds into 100 ml. of benzene maintained near the boiling point on a steam bath, drained momentarily, and dipped twice more. The series of three immersions was repeated with a fresh portion of hot benzene. After filtration through fritted glass funnels, the solutions were evaporated to dryness, cooled, and weighed. About 85 to 90% of the easily extractable material was recovered in the first solution and about 10% in the second.

On the three samples tested with a third portion of benzene, the amount of material recovered was less than 0.01% of the sample.

It is evident that two series of immersions made a sharp separation between the easily extractable material, mostly wax, of the surface and outer layers and the true oil from the interior of the grain.

After completion of the analysis for vitamins, oil, and nitrogen, composite samples of bran and of germ were made by combining all the remaining portions of these fractions. The results of starch and ash determinations on these samples do not represent the average composition of the five varieties, since the varieties did not contribute equally to the composite, but are indicative of the amounts of these constituents present.

Vitamin assays were made by microbiological methods. Riboflavin was determined by the method of Snell and Strong (12) following hydrolysis of the sample in 0.1 *N* hydrochloric acid at 120°C. for 30 minutes and filtration after neutralization to pH 6.8. Pantothenic acid was determined by the method of Skeggs and Wright (11). Biotin and pyridoxine were liberated by autoclaving at 120°C. for 30 minutes in the presence of 5 *N* sulfuric acid; biotin was determined in the neutralized filtrates by the method of Wright and Skeggs (16). The same filtrates were assayed for pyridoxine by the method of Atkin *et al.* (2).

Discussion

The physical analysis and composition of the whole grain and the fractions are given in Table I. The proportion of endosperm in the sorghum varieties separated ranged from 80.0 to 84.6%; the germ from 7.8 to 12.1%; and the bran 7.3 to 9.3%. These results are remarkably similar to those from the 11 samples of corn reported by Earle, Curtis, and Hubbard (6) which ranged from 79.7 to 83.5% endosperm, 10.2 to 14.1% germ, and 4.4 to 6.2% bran, and about 1% tip cap, and are in good agreement with the work of Bidwell (3) and Bidwell, Bopst, and Bowling (4).

In general, sorghum grain and its fractions closely resemble corn in the proportions of starch, protein, oil, and ash. Protein and starch are about 2% higher in the whole sorghum grain than in corn. In the

germ, the oil content is about 6% lower and the starch 5% higher than in corn germ. Microscopic examination of the separated germ fraction showed that some of the starch, perhaps a third, resulted from incomplete separation of the endosperm. Bran is likewise high in starch but,

TABLE I

COMPOSITION OF SORGHUM FRACTIONS AND WHOLE GRAIN (MOISTURE-FREE BASIS)

Fraction and variety	Per cent of whole kernel	Chemical composition					Vitamin content				
		Ash	Protein	Crude wax	Oil [1]	Starch	Niacin	Pantothenic acid	Riboflavin	Biotin	Pyridoxine
		%	%	%	%	%	μg./g.	μg./g.	μg./g.	μg./g.	μg./g.
Whole Grain											
Westland		1.67	13.2	0.29	3.5	72.3	47.8	15.5	1.4	0.22	6.2
Midland		1.57	12.0	.31	3.6	74.5	43.9	9.2	1.3	.19	4.9
Cody		1.68	12.4	.31	3.2	74.3	68.0	9.2	1.1	.18	4.2
Pink Kafir		1.67	12.3	.44	3.9	73.0	38.8	9.0	2.0	.23	4.2
Martin		1.57	11.5	.24	3.7	75.1	28.1	9.0	.8	.20	4.0
Average		1.65	12.3	0.32	3.6	73.8	45.3	10.4	1.3	0.20	4.7
Endosperm											
Westland	81.1	0.44	13.0		0.6	82.2	42.1	10.9	1.1	0.11	4.7
Midland	84.1	.39	12.0		.6	83.0	44.6	7.8	1.3	.10	4.3
Cody	84.6	.41	12.3		.8	83.0	70.5	9.5	.9	.11	3.7
Pink Kafir	80.0	.30	12.8		.4	83.2	34.6	6.8	.7	.13	3.6
Martin	81.7	.30	11.2		.4	81.3	26.9	8.6	.5	.11	3.8
Average	82.3	0.37	12.3		0.6	82.5	43.7	8.7	0.9	0.11	4.0
Germ											
Westland	9.6		19.1		27.3		105.5	51.1	3.8	0.53	8.2
Midland	8.5		19.1		30.6		69.5	25.9	4.3	.70	7.6
Cody	7.8		18.0		28.6		108.3	28.2	3.8	.54	7.2
Pink Kafir	12.1		19.2		27.1		70.5	27.1	3.4	.46	6.7
Martin	11.0		19.0		26.9		49.8	28.7	4.0	.60	6.5
Average	9.8	10.36[2]	18.9		28.1	13.4[2]	80.7	32.2	3.9	0.57	7.2
Bran											
Westland	9.3		6.8		3.7		43.4	12.0	3.6	0.30	4.9
Midland	7.4		7.5		5.3		53.8	8.4	4.8	.37	5.1
Cody	7.6		7.6		4.9		62.6	11.3	4.7	.34	4.0
Pink Kafir	7.9		6.4		6.0		32.8	8.8	3.9	.42	4.0
Martin	7.3		5.2		4.4		27.4	9.3	2.8	.31	3.9
Average	7.9	2.02[2]	6.7		4.9	34.6[2]	44.0	10.0	4.0	0.35	4.4

[1] Oil in bran and whole grain includes wax.
[2] Analysis of composite, not true average.

in this case, microscopic examination showed that under the tempering and soaking conditions used, the bran separated within the starchy mesocarp. Accordingly, the bran consisted of the cuticle, epidermis, hypoderm, and the major portion of the mesocarp. The innermost

fragments of the mesocarp, the nucellar layer, and aleurone remained with the endosperm fraction (13, 15). This is in contrast to corn, in which the microscope shows no starch within the bran cells. Sorghum bran is higher in oil (petroleum ether extract) than corn bran, but the extract consists mostly of wax rather than oil. The extract is semi-solid at room temperature and can be processed to give a hard wax (9).

TABLE II

PROPORTION OF THE TOTAL OF THE INDICATED CONSTITUENT EXISTING IN THE SPECIFIED FRACTION (CALCULATED FROM TABLE I)

Fraction and variety	Constituents								
	Ash	Protein	Oil [1]	Starch	Niacin	Pantothenic acid	Riboflavin	Biotin	Pyridoxine
	%	%	%	%	%	%	%	%	%
Endosperm									
Westland		81.1	14.1		70.7	59.5	56.3	52.6	75.3
Midland		82.2	14.4		79.3	70.0	60.9	49.4	78.0
Cody		84.0	20.6		81.9	72.3	54.2	57.4	78.5
Pink Kafir		78.3	7.9		71.3	57.6	43.4	53.6	71.5
Martin		78.7	9.1		74.6	64.7	37.6	50.6	75.5
Average	20.6[2]	80.9	13.2	94.4[2]	75.6	64.8	50.5	52.7	75.8
Germ									
Westland		14.1	75.9		21.0	33.0	22.9	30.7	15.6
Midland		13.2	74.4		12.5	23.4	19.9	34.6	13.9
Cody		11.3	68.0		11.6	19.9	20.8	26.5	13.9
Pink Kafir		17.8	80.5		22.0	35.0	32.5	29.2	20.5
Martin		18.0	82.0		18.6	29.1	42.5	36.8	17.5
Average	68.6[2]	14.9	76.2	1.8[2]	17.1	28.1	27.7	31.6	16.3
Bran									
Westland		4.8	10.0		8.3	7.5	20.8	16.7	9.1
Midland		4.6	11.2		8.2	6.6	19.2	15.9	8.1
Cody		4.7	11.4		6.5	7.8	25.0	16.1	7.6
Pink Kafir		3.9	11.6		6.7	7.4	24.1	17.2	8.0
Martin		3.3	8.9		6.8	6.2	19.9	12.6	7.0
Average	10.8[2]	4.0	10.6	3.8[2]	7.3	7.1	21.8	15.7	8.0

[1] Oil in bran includes wax.
[2] Calculated from analysis of composite sample.

Vitamin content varies much more among varieties than does the proportion of major constituents. Since the analyses of Tanner, Pfeiffer, and Curtis (14) show about twice the range in vitamin content reported here, it would seem that five samples are inadequate for a completely satisfactory picture. However, the general relationship between the composition of the fractions is probably shown satisfactorily by the five samples. For example, analysis of the germ of numerous varieties would probably extend the range of vitamin content

found in the germ, but would not change the conclusion that the germ is the richest of the fractions and contains in general a concentration from two to five times as high as that of the endosperm and bran. Riboflavin in bran is exceptionally high, equaling that in germ. Niacin, pantothenic acid, and pyridoxine occur in the bran and endosperm in essentially the same concentration.

Table II shows the proportion of each constituent which occurs in the various fractions. Five samples are probably sufficient also to give a satisfactory representation of this distribution.

Acknowledgment

The authors are indebted to Miss Margaret Shekleton and Mrs. Irma Bolam for their assistance in the analyses of these samples.

Literature Cited

1. Association of Official Agricultural Chemists. Official and tentative methods of the Association of Official Agricultural Chemists. (6th ed.) Assoc. of Off. Agr. Chem.: Washington, D. C. (1945).
2. Atkin, L., Schultz, A. S., Williams, W. L., and Frey, C. N. Yeast microbiological methods for determination of vitamins. Pyridoxine. Ind. Eng. Chem. (Anal. Ed.) **15**: 141–144 (1943).
3. Bidwell, G. L. The physical and chemical study of the kafir kernel. U. S. Dept. Agr. Bull. **634**: 6 (1918).
4. Bidwell, G. L., Bopst, L. E., and Bowling, J. D. A physical and chemical study of milo and feterita kernels. U. S. Dept. Agr. Bull. 1129: 1–8 (1922).
5. Clendenning, K. A. Polarimetric determination of starch in cereal products. IV. Critical studies of methods for the determination of starch in whole-wheat, granular and patent flours. Can. J. Research **B23**: 239–259 (1945).
6. Earle, F. R., Curtis, J. J., and Hubbard, J. E. Composition of the component parts of the corn kernel. Cereal Chem. **23**: 504–511 (1946).
7. Edwards, W. M., and Curtis, J. J. Grain sorghums, their products and uses. U. S. Dept. Agr., Bur. Agr. Ind. Chem. ACE-193, NM-229, Northern Regional Research Laboratory: Peoria, Ill. (1943).
8. Hightower, H. V. Wet milling advances a century—in a decade. Food Ind. **21** (8): 40–44 (1949).
9. Kummerow, F. A. The composition of sorghum grain oil, andropogon sorghum var. vulgaris. Oil and Soap **23**: 167–170 (1946).
10. Ross, W. H., and Hardesty, J. O. Grinding of fertilizer samples for analysis. J. Assoc. Agr. Chem. **25**: 238–246 (1942).
11. Skeggs, Helen R., and Wright, L. D. The use of *Lactobacillus arabinosus* in the microbiological determination of pantothenic acid. J. Biol. Chem. **156**: 21–26 (1944).
12. Snell, E. E., and Strong, F. M. A microbiological assay for riboflavin. Ind. Eng. Chem. (Anal. Ed.) **11**: 346–350 (1939).
13. Swanson, A. F. Seed-coat structure and inheritance of seed color in sorghums. J. Agr. Res. **37**: 577–588 (1928).
14. Tanner, F. W., Jr., Pfeiffer, Shirley E., and Curtis, J. J. B-complex vitamins in grain sorghums. Cereal Chem. **24**: 268–274 (1947).
15. Winton, A. L. The anatomy of the fruit of certain cultivated sorghums. Conn. State Agr. Expt. Sta. Ann. Rept. (1902) **26**: 326–338 (1903).
16. Wright, L. D., and Skeggs, Helen R. Determination of biotin with *Lactobacillus arabinosus*. Proc. Soc. Expt. Biol. Med. **56**: 95–98 (1944).

Cereal Chemistry

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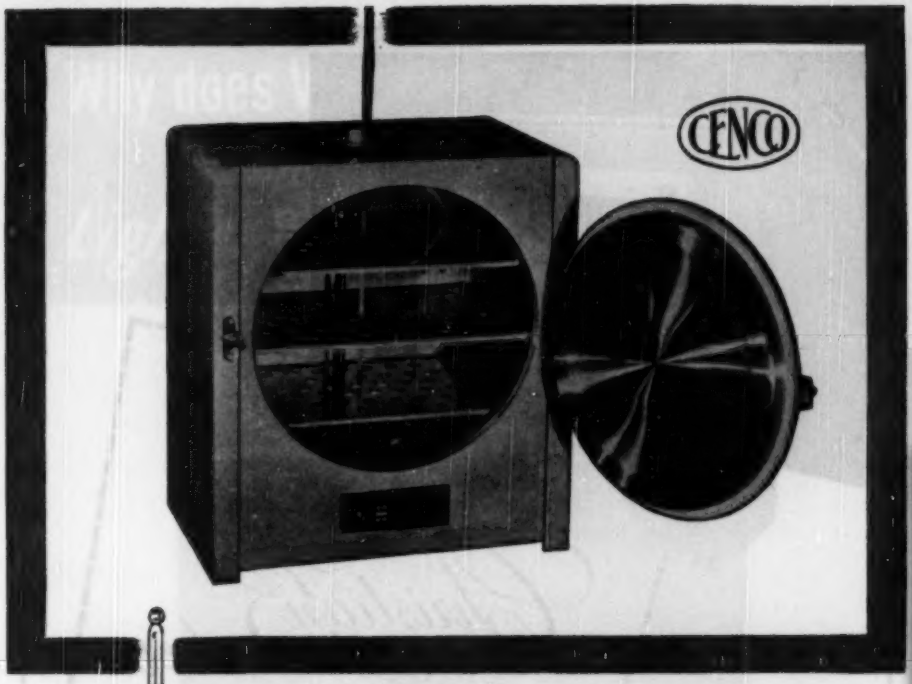

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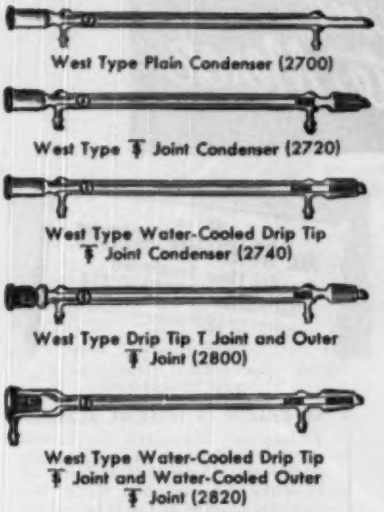

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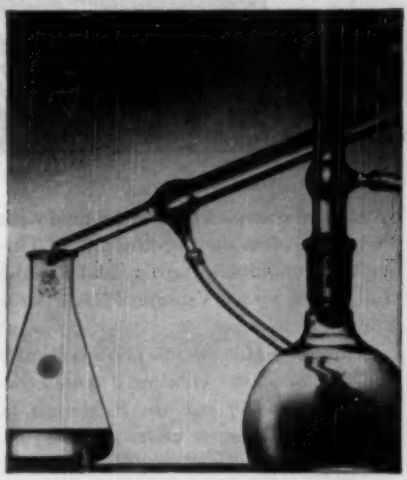

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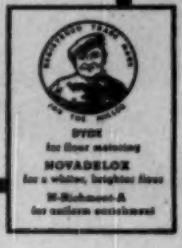

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